Breeding and Seed Production of Chinese Cabbage in the Tropics and Subtropics
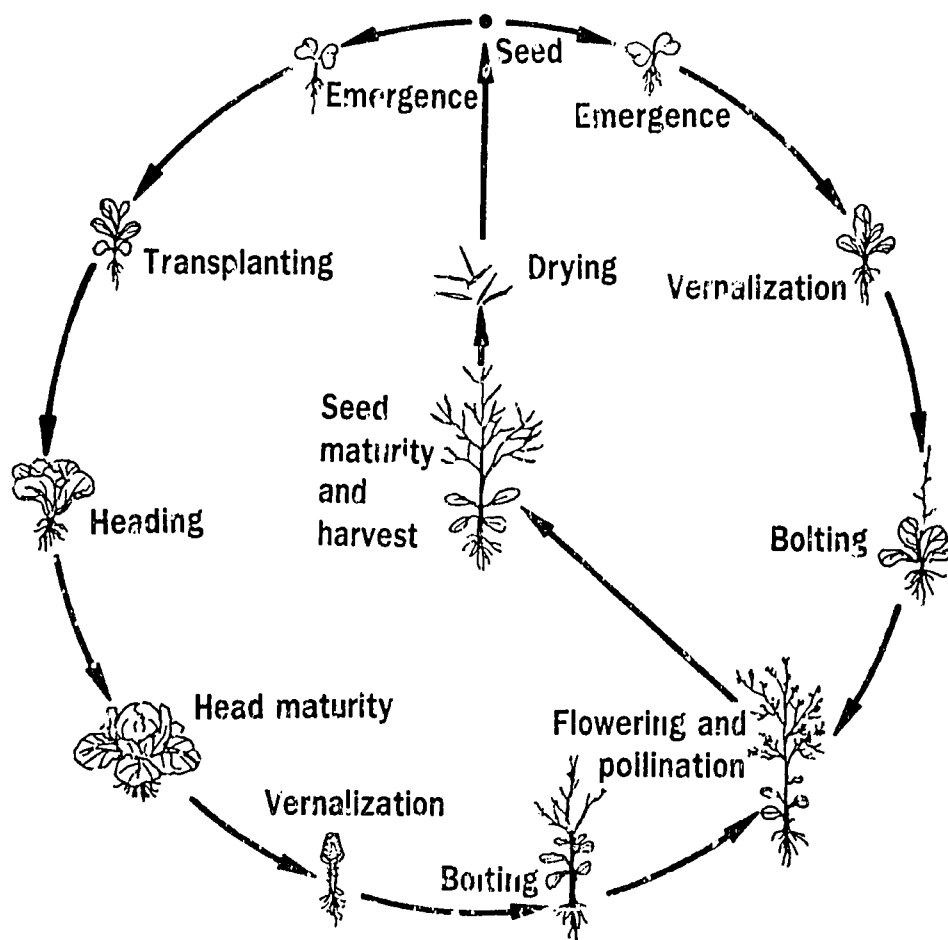
R.T. Opeña, C.G. Kuo and J.Y. Yoon

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Acknowledgment

Preface

Heading Chinese cabbage (*Brassica campestris* subsp. *pekinensis*) is traditionally a crop of the temperate zones where it grows well under cool, dry climate. It is most popular in temperate East Asia where it ranks as one of the most important vegetables. In the tropics the production of heading Chinese cabbage is confined mainly to the highland areas. When grown in the lowland tropics, this vegetable fails to form marketable heads and easily succumbs to several debilitating diseases.

The successful growing of heading Chinese cabbage in the hot, humid tropics was not feasible until the advent of the heat-tolerant, tropically-adapted cultivars. These improved varieties resulted from the painstaking crop improvement research which commenced in 1972 at the Asian Vegetable Research and Development Center (AVRDC). In 1980 AVRDC cosponsored the First International Symposium on Chinese Cabbage in Tsukuba, Japan, in part to herald the new horizon for this vegetable in the tropics. At that time the issue of genetically adapting the heading Chinese cabbage to high temperature conditions was no longer moot — AVRDC research had established this unequivocally.

Further development of improved technology beyond the laboratories and experimental fields of AVRDC has been, and still is, the legitimate concern of the national programs. The testing and adoption of AVRDC's new Chinese cabbage cultivars specifically bred for the tropics was no exception. In order to help the national programs to achieve this, AVRDC intensified the distribution of sample seeds of its improved cultivars in the years following the Tsukuba symposium. Exhaustive tests by national program scientists and by other interested parties amply demonstrated the feasibility of growing heading Chinese cabbage in the hot, humid tropics. Some national programs in the tropical region have officially released the improved genetic materials to their vegetable farmers; in others, performance of the new cultivars warranted their commercial release if not for the problem of inadequate seed supply that many national program authorities anticipated and were correctly apprehensive about.

The seed production dilemma in the tropics is not one without a technological solution, however. While Chinese cabbage is largely 'temperate' in its requirements for flowering and seed production, the tropically adapted cultivars do not require very low temperatures for flowering induction. Successful seed production of these cultivars has been demonstrated in the highlands of the Philippines and Indonesia. Indeed, it is not farfetched to expect that other tropical countries interested in developing their own seed production program for the tropical cultivars could very easily do so given the following: availability of relatively cool, dry highland climate; trained and skilled cadre of seed production specialists; and, appropriate technology for seed production.

AVRDC has been actively assisting some interested national programs in developing their own Chinese cabbage seed production capabilities. Already, a few specialists from these national programs have completed the research internship training at AVRDC on Chinese cabbage seed production, with special emphasis on the multiplication of Hybrid No. 62, the most widely adapted of the new cultivars.

The present bulletin was prepared as part of AVRDC's continuing commitment to technology transfer to the national programs. It is intended mainly to provide the seed production specialists in the national programs with the fundamental guidelines for producing Chinese cabbage seeds in the tropics. We feel strongly that a good working knowledge of plant breeding is essential for a better understanding of the general seed production principles and practices for any crop. In this bulletin, therefore, we have also aimed at imparting the basic principles which underlie

the breeding of a cross-pollinated crop like Chinese cabbage; more specifically, its improvement for tolerance to the major stresses of the humid tropics was an important consideration in this publication. This bulletin could also serve as a special reference on Chinese cabbage, especially in the aspects of its evolution, its biosystematic relationship with the other *Brassica* species, its general botany, the genetics and physiology of self-incompatibility, and other related subjects.

References to the other *Brassica* species have been made frequently in this bulletin to illustrate a point or to expound on a particular topic in which the required information from Chinese cabbage is fragmentary. These citations hopefully will not serve to detract on the principal topic of this bulletin — Chinese cabbage in the tropics and subtropics. This is a very gray area as far as information is concerned. By documenting the experiences and the results of research at AVRDC to make the Chinese cabbage a veritable tropical crop, we are optimistic that we are sharing a useful body of knowledge that others could benefit from.

This bulletin is the culmination of over a decade long research endeavor by all AVRDC scientists to bring the Chinese cabbage within reach of tropical vegetable farmers. This technological feat has been accomplished. To avail the seeds to the tropical farmers has been a long time coming. Delivering the technology of seed production to the national program through this bulletin is one small but vital step in this process.

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Introduction

Importance of Chinese Cabbage

Heading Chinese cabbage is one of the most important vegetables in eastern Asia. In China it is the most widely grown among 100 or so commonly cultivated vegetables. In the northern areas it accounts for more than one-fourth of the total annual vegetable consumption. In Japan it is also very popular, ranking third after radish and cabbage in annual production. Farmers in almost every prefecture cultivate the crop, and it is grown on a total of at least 35,000 ha. In Korea it is undoubtedly the most important vegetable both in terms of production area and per capita consumption. More than 90% of the Chinese cabbage harvested in Korea is used for making 'kimchi', a fermented side dish eaten all year-round by virtually every family. In Taiwan Chinese cabbage is very popular; an annual production area of 9,000 ha is second only to common cabbage.

Chinese cabbage is favored by small farmers in eastern Asia because of its short cropping duration. It is an efficient food producer, a good cash crop, an appetizing food item, and a valuable source of calcium, crude fiber, and vitamin C in the human diet (Table 1). In the tropics and subtropics, there has been a considerable increase in production in the past decade because of the availability of new, tropically adapted varieties. However, the bulk of the Chinese cabbage seeds in the tropics and subtropics are still imported. With the much needed boost in the development of seed production technology in the tropics and subtropics in recent years, production of this crop is expected to expand significantly in the tropical lowland areas.

Table 1. Nutrient composition of some *Brassica* vegetables (in 100 g of edible portion).[z]

Vegetable	Moisture (%)	Protein (g)	Fiber (g)	Calcium (mg)	Iron (mg)	Vitamin A (μg carotene eq)	Vitamin C (mg)
B. campestris subsp. chinensis	94.2	1.7	0.7	102	2.6	2,305	53
B. campestris subsp. parachinensis	93.7	2.1	0.8	109	3.1	2,045	60
B. campestris subsp. pekinensis	95.0	1.4	0.6	49	0.7	890	38
B. oleracea var. capitata	93.0	1.6	0.8	55	0.8	280	46
B. oleracea var. gemmifera	85.2	3.9	1.6	40	1.4	50	71
B. oleracea var. italica	89.1	3.4	0.8	86	1.4	685	111
B. oleracea var. botrytis	90.5	2.8	0.9	30	1.0	55	72
B. juncea	91.8	2.4	1.0	160	2.7	1,825	73

[z] Food Composition Table for Use in East Asia', 1972. Food and Agriculture Organization of the United Nations, and U.S. Dept. of Health, Education, and Welfare.

Origin and Distribution of Chinese Cabbage

Heading Chinese cabbage is a member of the genus *Brassica* and a native of eastern Asia. Its progenitor form, *B. campestris*, is believed to have evolved in the Mediterranean area and later been introduced to northern Europe where it was improved as an oil seed crop (Nishi 1980). After introduction to China more than 2,000 years ago, it differentiated into various subspecies (Lee 1982). Both *B. campestris* subsp. *rapa* (turnip) and *B. juncea* (mustard) were recorded in the fifth century B.C. in China. Until the seventh century A.D., *B. campestris* subsp. *rapa* was grown only in north China. On the other hand, *B. campestris* subsp. *chinensis* (nonheading

Chinese cabbage) was cultivated only in south China, at least for 1,600 years. The loose-leaved heading Chinese cabbage (*B. campestris* subsp. *pekinensis*) was first illustrated in the 10th century A.D., and a clear illustration of the heading shape of Chinese cabbage with wrapping leaves was first recorded in 1330 (Jiang 1981, Li 1981). Much of the evolution of heading Chinese cabbage took place in China from about 600 years ago. Li (1981) suggested that *B. campestris* subsp. *pekinensis* might have originated in central China where subsp. *rapa* and subsp. *chinensis* were commonly grown together and had ample opportunity to interbreed.

The first record of Chinese cabbage in Korea dates back to the 13th century. However, heading Chinese cabbage did not become one of Korea's most important vegetables until the 19th century (Pyo 1981). *B. campestris* subsp. *pekinensis* was first introduced into Japan from China in 1866. It was not until 1920 that the stage was set for cultivar development of Chinese cabbage in Japan (Watanabe 1981).

Nonheading *B. campestris* subsp. *chinensis* was introduced to the Strait of Malacca in the 15th century. Recently, it has become popular in Malaysia, Indonesia, and western India. On the other hand, heading *B. campestris* subsp. *pekinensis* is a late introduction to Southeast Asia. It is grown mostly during the cool, dry season in the subtropical lowlands or throughout the year in the tropical highland areas. With the recent development of heat-tolerant varieties, production of this crop has become feasible in the tropical lowland areas.

The cultivation and peculiarities of *B. campestris* subsp. *pekinensis* were described in France as long ago as 1840 by Pepin, who said that, 'while the plant had been known in the botanical gardens for 20 years, it was brought to notice as a culinary vegetable only three years before' (Bailey 1928). It was first introduced to England in 1887. In the USA it began to attract attention about 1883, and was first grown by L. H. Bailey in 1893 using a seed from Kew, England.

In recent years *B. campestris* subsp. *pekinensis* and subsp. *chinensis* have spread widely outside Asia, mainly through oriental immigration to western nations. Chinese cabbage is gaining more popularity among the non-oriental people especially in North America and western Europe. It is now becoming firmly established as a vegetable crop in many parts of the temperate region.

Biosystematic Relationship among the *Brassica* Species

The genus *Brassica* is comprised of several economically important species which yield edible roots, stems, leaves, buds, flowers and seeds; some are used as forage and oil seed crops. The taxonomy of the *Brassica* is complicated. This genus is comprised of six species; three are considered basic species and the rest are amphidiploid forms (Fig. 1). The three elementary species, their chromosome numbers, and genomic nomenclatures are: *B. nigra* Koch (black mustard, n = 8, genome B); *B. oleracea* L. (cole crops, n = 9, genome C); and *B. campestris* L. (turnip and Chinese cabbage group, n = 10, genome A). The amphidiploids are: *B. carinata* Braun (Ethiopian mustard, n = 17, genome BC). *B. napus* L. (swedes, rape, rutabagas, n = 19, genome AC), and *B. juncea* (L.) Czern. (brown mustard, n = 18, genome AB). Evidently, they originated in nature from crosses between the elementary species. Among those with 9, 10 and 18 chromosomes, there are many subspecies, botanical varieties or groups (Table 2). Those with 10 or 18 chromosomes, along with *Raphanus sativus* L. (radish, n = 9, genome R), are the forms that are extensively grown in Asia. There is a wide differentiation of varieties (Nishi 1980).

The heading Chinese cabbage, often referred to simply as *B. pekinensis*, actually belongs to the basic *B. campestris* species. A more appropriate Latin name for heading Chinese cabbage is *B. campestris* subsp. *pekinensis*. The 10-chromosome group (*B. campestris*) includes the turnip (root), oil seed rape, and the Chinese cabbages (Color plate A, p. 42). Much has been written about the systematic position of these plants (Cao and Li 1982, Lee 1982, Lin 1980, Nishi 1980, Vaughan 1977). Generally, *B. campestris* could be subdivided into several subspecies. Some of the major ones are described below.

Brassica campestris subsp. *campestris*. This subspecies is considered the most primitive among the leafy vegetable forms of *B. campestris*. Its flowering stalks and rosette leaves are

Table 2. Common vegetables and some related varietal or subspecific taxa of the genus *Brassica*.

Brassica sp. (n)	Subsp. or var.	Common name
campestris (10)	subsp. *chinensis*	pak choi
	subsp. *narinosa*	broad beaked mustard
	subsp. *nipposinica*	
	subsp. *oleifera*	turnip rape
	subsp. *parachinensis*	choy sum
	subsp. *pekinensis*	Chinese cabbage
	subsp. *perviridis*	mustard spinach, tendergreen
	subsp. *rapifera* or *rapa*	turnip
	subsp *trilocularis*	sarson
	subsp. *utilis*	
juncea (18)	var. *capitata*	head mustard
	var. *crispifolia*	cut leaf mustard
	var. *faciliflora*	broccoli mustard
	var. *lapitata*	large petiole mustard
	var. *multiceps*	multishoot mustard
	var. *oleifera*	oil seed mustard
	var. *rapifera*	root mustard
	var. *rugosa*	leaf mustard
	var. *spicea*	mustard
	var. *tsa-tsai*	big stem mustard
oleracea (9)	var. *acephala*	kales
	var. *alboglabra*	Chinese kale, kailaan
	var. *botrytis*	cauliflower, heading broccoli
	var *capitata*	cabbage
	var. *costata*	Portuguese cabbage
	var. *gemmifera*	brussel sprouts
	var. *gongylodes*	kohl rabi
	var. *italica*	broccoli, calabrese
	var. *medullosa*	marrow stem kale
	var. *palmifolia*	kale, Jersey kale
	var. *ramosa*	thousand-head kale
	var. *sabauna*	savoy cabbage
	var. *sabellica*	collards
	var. *selensia*	borecole

Sources: Anonymous 1985; Terrell et al. 1986.

edible. This subspecies is not far evolved from the oil seed rapes of China which are supposed to be the common progenitor of all the leafy vegetables of *B. campestris* (except subsp. *japonica* which may have had a history of introgression from mustard — *B. juncea*). In India *B. campestris* and its derivative (commonly called 'sarson') are grown as an oil seed crop.

Brassica campestris subsp. chinensis. This subspecies is commonly referred to also as Chinese cabbage. This group is made up mainly of nonheading types. Where heading Chinese cabbage cannot be grown owing to unsuitable environment for head formation, the nonheading types are often popular. There are several recognized types: narrow petioles with round cross section vs. wide petioles with flat cross section, white vs. green petioles, variation in size, foliage color, etc. Varietal differentiation in this subspecies has been remarkably more pronounced than in any other leafy vegetables of the genus *Brassica*.

Brassica campestris subsp. japonica. This subspecies is one of the unique vegetables of Japan. This subspecies is typically characterized by an excess of basal branches and leaves.
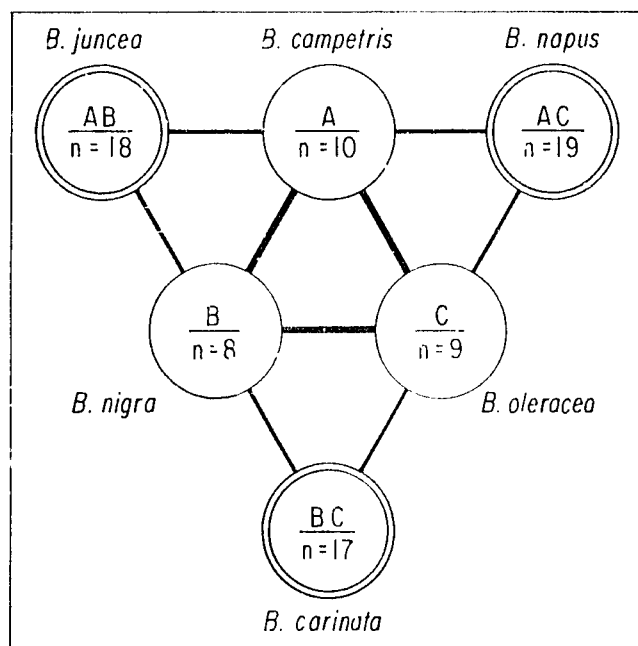
Figure 1.
Genomic relationship of *Brassica* taxa based on chromosome numbers. (Species with single circles are monogenomic, while those with double circles are amphidiploids).

Two types are distinguished: 'Mizuna', with deeply dissected, bipinnate leaves and 'Mibuna', with slender entire leaves. The formation of subsp. *japonica* is thought to have involved some introgression from the mustard group, *B. juncea* (Matsumura 1954). This vegetable resembles *B. juncea* in petiole and silique conformation, and seed size; the flower stalks of both are not enclosed by leaves.

Brassica campestris ssp. narinosa. This subspecies is commonly known as Chinese flat cabbage. The plant is generally low, compact and stout, producing clusters of thick, often wrinkled leaves with broad, white petioles. This subspecies is well known for its cold tolerance. The crisp leaves and thick petioles are excellent for preparation as a boiled vegetable.

Brassica campestris subsp. oleifera. This subspecies is an oil-yielding crop. The plants have many branches, with exceedingly well-developed siliques and seeds.

Brassica campestris subsp. parachinensis. This subspecies is referred to as flowering Chinese cabbage and considered a derivative of subsp. *chinensis* because of similarities in petiole morphology. However, subsp. *parachinensis* readily bolts and branches profusely from the leaf axils. The flowering stalks constitute the main edible portion.

Brassica campestris subsp. pekinensis. This subspecies comprises mainly heading types of Chinese cabbage. Heads are marketable parts; they vary in degree of compactness and may be divided into loose, semiheading, and completely heading types. Further variation in head shape can be noted, e.g. long, short, tapered, round or flat top, wrapped-over or joined-up leaves, etc.

Brassica campestris subsp. rapa or rapifera. This subspecies is commonly called turnip. This group is cultivated either as a vegetable or fodder. They grow best in a cool climate and many diverse ecotypes occur, especially in Japan which is considered one of the major centers of varietal development (Nishi 1980). Since the cultivation and utilization of turnips often overlap with radishes, the greater productivity of the latter reduces the relative importance of turnips. Classification of world turnips into different types have been proposed by several workers (Nishi 1980, Sinskaja 1928, Vaughan 1977). Generally, Asian types can be distinguished from European

types because leaves of the former tend to be entire and glabrous. The well-developed tap root, the edible part of Asian cultivars, indicates the great improvements made during its history of cultivation.

Evolution and Variation in Chinese Cabbage

Although *B. campestris* originated in the Mediterranean area and was only introduced to Asia through northern Europe, the most significant changes in form, structure, and productivity in this species have occurred in eastern Asia through artificial albeit perhaps unconscious, selection by growers. As noted earlier, there are two main groups of Chinese cabbage: heading group or Pe-tsai (*B. campestris* subsp. *pekinensis*), and the nonheading group or Pak-choi or Bok-choy (*B. campestris* subsp. *chinensis*). The names Pe-tsai and Pak-choi (Bok-choy) both mean 'white vegetable' in Chinese languages; Pe-tsai is the Beijing (Peking) or Mandarin dialect, while Pak-choi (Bok-choy) is the Cantonese dialect. Choi-sum, which means 'vegetable heart' in the Cantonese dialect, is a flowering Chinese cabbage (*B. campestris* subsp. *parachinensis*). Many other synonyms and spellings exist for these groups of Chinese cabbage in Chinese languages (Table 3). For formal differentiation, Chinese cabbage is referred to as *B. campestris* subsp. *pekinensis* in this bulletin.

Table 3. Various vernacular names and spellings of Chinese cabbages in two Chinese dialects.

Subspecies	Cantonese	Beijing or Peking[1]	
		Pinyin	Wade-Giles
subsp. chinensis	pak-choi, bok-choy, pak-tsoi, paak-tsoi, or baak-cho. (white veg.)	xiao-bai-cai (small white veg.), bai-cai (white veg.), qing-cai (green veg.)	pe (or pan)-tsai (white veg.), hsiao (or ...) pe-tsai (small white veg.
subsp. parachinensis	choi-sum or tsoi-sum (veg heart), paak tsoi sum (white veg. heart)	cai-xin (veg. heart), cai-tai (veg. brassica), cai-gian (veg. tip)	tsai-hsin (or sin) (veg. heart), tsai-tai (veg. brassica)
subsp. pekinensis	wong-nga-pak (yellow sprouted white), shu-tsoi (shu veg.), wong-bok (yellow white)	da-bai-cai (big white veg.), jie-jiu-bai-cai (heading white veg.)	chieh-chiu-pe-tsai (heading white veg.), pao-hsin (or sin) -pai-tsai (wrapped heart white veg.), ta-pe (or pai)-tsai (big white veg.)

[1] The Beijing or Peking dialect is an official language in China and Taiwan, Pinyin romanization is used in China and Wade-Giles romanization in Taiwan, respectively.

Heading Chinese cabbage is believed to have, as its primitive form, a loose-leaved (or nonheading) type from which progressively compact head types further evolved. Li (1981) hypothesized that the elementary cultivated form, a loose-leaved variety which he called var. *dissoluta*, was probably produced through the natural hybridization between Pak-choi (*B. campestris* subsp. *chinensis*) and another subspecies of *B. campestris*, the turnip (*B. campestris* subsp. *rapifera*). This hybrid evolved successively into a semiheading variety, then to a fluffy-topped heading variety and finally into the completely heading type. In Japan the completely heading sorts have further differentiated into various types mainly through breeders' intervention (Watanabe 1981).

According to the shape, size, and organization of the head, there are three principal patterns belonging to three varieties as described below (Tsen and Lee 1942, Lee 1984, Li 1981). Numerous varieties have been further developed from these three varieties (Fig. 2).
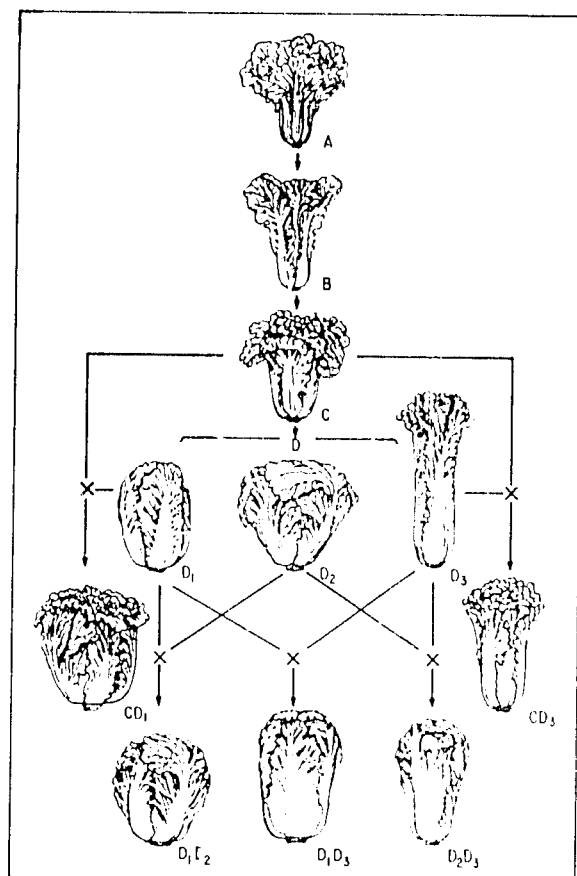
Figure 2.
The evolution of Chinese cabbage
(*Brassica campestris* subsp. *pekinensis*): (a.)
var. *dissoluta*; (b.) var. *infarcta*; (c.) var.
laxa; (d.) var. *cephalata*, D_1. f. *ovata*,
D_2. f. *depressa*, D_3. f. *cylindrica*, CD_1.
var. *laxa* xf. *ovata*, CD_3. var. *laxa* xf.
cylindrica, D_1D_2. f. *ovata* xf. *depressa*,
D_1D_3. f. *ovata* xf. *cylindrica*, D_2D_3. f.
depressa xf. *cylindrica* (from Li 1981).

Brassica campestris var. cephalata. This variety is the most common pattern of heading Chinese cabbage. The large compact heads may be ovate or obovate in shape. The head leaves curve inward and overlap at the top. Head shape can be classified into round, ovoid, and flattened types.

Brassica campestris var. cylindrica. This variety forms a compact head which is erect and elongated in shape, and with or without heading leaves over the top. The heads are more or less pointed at the top and spirally wrapped.

Brassica campestris var. laxa. This variety forms a loose, open head, known as 'flowery hearted type'. The tip and the upper margins of the head head leaves may be erect or curled outward, and are yellow or yellow-white in color.

In addition to the variation in head type, the subsp. *pekinensis* shows tremendous differences in other important traits such as maturity, head weight, head compactness, leaf number, and leaf color (Lee 1984, Watanabe 1981). The period from sowing to head maturity of heading Chinese cabbage can range from 55 to over 110 days under favorable conditions depending upon the genotype; within this range varieties can be divided into different maturity groups. Plant weight, excluding the root part, can be as light as a few hundred grams in some varieties, and as heavy as 10 kg or over at the other extreme. Leaf number may be as few as 20 and as numerous as 150. A considerable variation with respect to bolting and head formation has also been recognized in response to temperature. The available variations in the above attributes are essential to the genetic improvement of heading Chinese cabbage.

General Botany and Reproductive Biology

Development and Growth Stages

Chinese cabbage plants pass through various growth phases or stages in their development. The growth stages are among the most important variables in assessing varietal characteristics, maturity, yield potential, the impact of disease or insect damage, seed-production potential, etc. In designing useful growth stages, the description of the various phases must correspond to clearly recognizable features of the species (Fig. 3). The following stages, although not formally recognized, are frequently used to describe the various growth phases of Chinese cabbage. The actual time it takes to develop from one stage to another depends on variety, weather, and management practices.
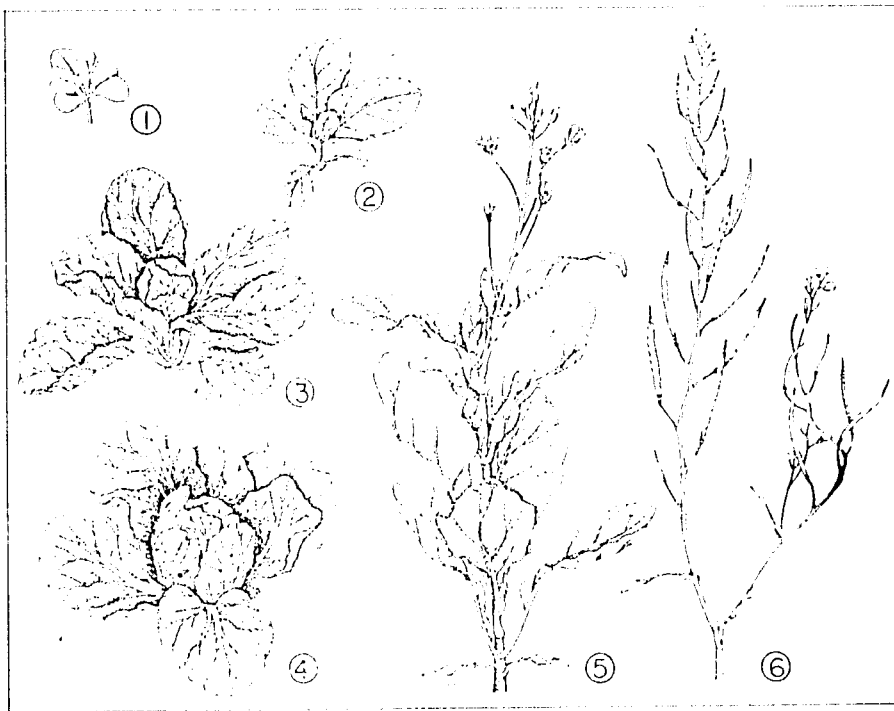
Figure 3. Growth stages of Chinese cabbage for crop and seed production purposes: (1) emergence stage; (2) seedling stage; (3) heading stage; (4) harvesting stage; (5) flowering stage; and (6) silique and seed stage.

Emergence stage. Germination of seeds requires water, oxygen, and suitable temperatures. Once Chinese cabbage seeds absorb water up to a 40% to 50% moisture content, they germinate rapidly. The radicle first emerges out of the seed, usually about 24 hours after the seed has

taken in water under optimum temperatures. After the young root has grown 2-3 cm into the soil, the seedling begins to grow upward. The hypocotyl emerges first at the soil surface, and the two cotyledons unfold at the top of the hypocotyl, and then part and extend. In optimum environments Chinese cabbage seedlings take three to four days to emerge above the soil.

Seedling stage. The first two true leaves develop between the fully extended cotyledons, and the plant begins to carry out photosynthesis. Later, many leaves are formed at the growing point without much increment in height. There are normally five leaves in two whorls among early-maturing varieties, and eight leaves in three whorls among late-maturing ones. Transplants are usually set out to the field at this stage.

Rosette stage. The first two or three whorls of the leaves are fully expanded into rosette, nonheading leaves in more or less horizontal position and close to the soil surface. New leaves are continually formed at the growing point. The inner leaves start to grow more upright, usually under shaded conditions, after new whorls of leaves have grown.

Heading stage. The crop assumes its marketable shape at this stage. Heading begins at about the 12th to 13th leaf stage for early-maturing varieties or the 24th to 25th leaf stage for late-maturing ones when the youngest, innermost leaves start to incurve and touch at their tips. As new leaves form and expand around the vertical central axis of the plant, their margins become temporarily entrapped against the upright leaves. In the early stages of head formation, these temporarily entrapped leaves finally unfold, become upright, and roll outward to develop into the outer head leaves. As more leaves are produced in the center, they become increasingly entrapped until they remain folded in the center to form the compact mature head. There is a limited increase in height at this stage, but the plant assumes its characteristic heading shape. The young head grows fast and increases in size until the maximum size and firmness have been reached, and it is ready for harvest.

Flowering stage. Flower initiation takes place either before or after heading depending upon the temperature and/or photoperiod during the growth period. The stem normally elongates (bolts) as the flower buds initiate and develop.

Silique and seed stage. After fertilization, the endosperm and the siliques containing 10-25 seeds develop rapidly and reach their full length and diameter within three to four weeks. The fully developed siliques require about two weeks to mature.

Morphological Features

Root. Chinese cabbage is characterized by a very extensive, fibrous, finely branched root system. As the true leaves continue to grow, the taproot grows deep into the soil and the first lateral roots form. The primary taproot is usually no more prominent in the early stage than the other major laterals which arise in great numbers from the base of the enlarged underground part. At first, nearly the entire root system consists of widely spreading branches in the surface soil. These run obliquely downward, with the vertically descending laterals thoroughly occupying the deeper soil zone. The root system is extremely fine and fragile; most roots attain a thickness of no more than 0.5 mm. During early growth stages, direct-seeded plants have a longer taproot and are better anchored than transplants, whose root growth is mostly lateral because the taproot ends are often damaged during transplanting. Mature vegetative plants have a working level of 35 cm, to which depth the soil is well ramified with a profuse network of active rootlets. More than 90% of the root system of a mature vegetative plant are within a 35-cm-depth and 40-cm-diameter soil zone. When Chinese cabbage plants are uprooted and set out again, new roots begin to grow. The root system during the reproductive stage is much more developed than in the vegetative stage.

Stem. During the vegetative stage, the unbranched stem of Chinese cabbage generally remains less than 20 cm long owing to a restricted and lengthwise growth at the early stage. At this time, however, the stem continues to thicken. The diameter at the stem base may reach 4 to 7 cm and the stem has a well-developed pith. As the plant reaches the reproductive stage, the growing point of the stem forms the floral primordia; its lower part starts to elongate and reaches 60 to 100 cm in length. There are primary, secondary, and tertiary branches; the lower branches are usually longer than the top branches.

Leaf. The leaf shape of Chinese cabbage changes with the growth stages, and various types can be distinguished as indicated below.

Cotyledon: Two notched, kidney-shaped cotyledons are stalked and opposite each other. The food stored in the cotyledons is used during the early development of the seedling; the cotyledons eventually decrease in size, wither and fall off.

Basal leaf: Two true leaves originate opposite one another at the same height, but perpendicular to the plane of the cotyledons, thus, forming a cross shape. Basal leaves are oblong, stalked, and 8 to 15 cm in length. The basal true leaves also drop off after a few weeks of growth.

Nonheading leaf: Leaves arise alternately on the axis of the enlarged, but compressed, stem. A spiral path of leaves may have five leaves in two whorls or eight leaves in three whorls. Leaves are mostly sessile. The leaf blade extends to the bottom of the midrib to form a wing shape. The leaf margin is wavy, but notched at the bottom of the leaf blade. The leaves of the first spiral path are small; they contribute to the growth of larger nonheading leaves. The rosette-shaped nonheading later whorls of leaves expand rapidly one after another, and are essential for head formation and differentiation of the inner leaves. These nonheading leaves aid head formation by supplying photosynthates to the inner leaves, and by establishing a posture for head formation, as well as providing shade to the head.

Head leaf: Head leaves also form on the top of the enlarged, but compressed, stem with a similar phyllotaxis as the rosette leaves. The outer head leaves on the periphery of the head are narrow and oval with long petioles, whereas the inner, entrapped leaves are broad and round with the ratio of leaf length to breadth approaching unity. The details of these changes are discussed later (see Growth and Patterns of Head, p.10).

Stem leaf: Leaves arise alternately on the flowering stem or branch. The petiole is broad and compressed, and wraps the flowering stem and branches. Leaves are lanceolate, much smaller than the nonheading and head leaves, and often noticeably smooth.

Inflorescence. A simple, elongated, indeterminate inflorescence bears stalked or pedicelled flowers in terminal racemes on the main stem and its branches. Individual flowers are supported by pedicels attached to the main axis of the inflorescence. The inflorescence may attain a length of ca. 1 m but the slender pedicels are only about 1-1.5 cm long.

Flower. Flowers are bisexual and perfect. During differentiation four sepals, six stamens, two carpels, and four petals successively develop (Fig. 4). The carpels form a superior ovary with a 'false' septum and two rows of campylotropous ovules. The androecium is tetradynamous, i.e. there are four long and two short stamens. The bright yellow petals are arranged in the form of a cross, thus the family name Cruciferae. The four sepals are more or less erect. The buds open under the pressure of the rapidly growing petals. The opening process begins in the afternoon and usually the flowers are fully expanded by the following morning. The anthers open a few hours later than the flowers, the latter being slightly protogynous. The nectar, which attracts pollinators — particularly bees, is secreted by the two nectaries situated between the
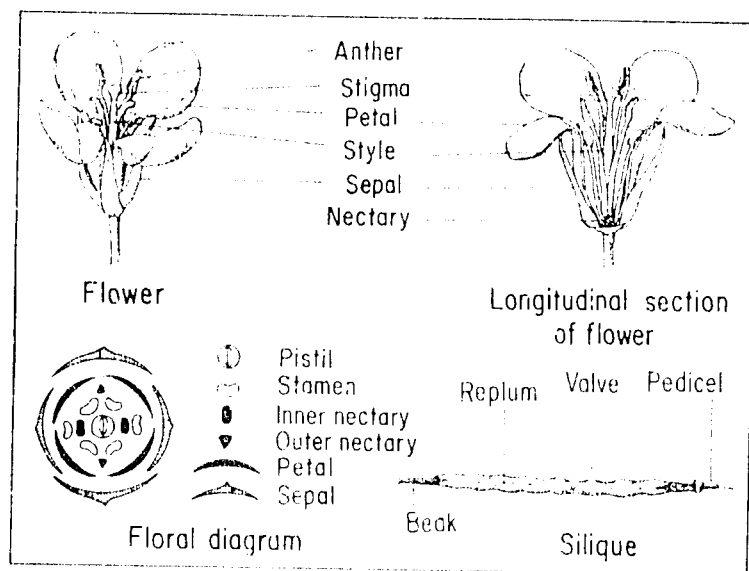
Figure 4.
Parts of Chinese cabbage flower and siliques.

bases of the short stamens and the ovary. Two other inactive nectaries are situated outside the bases of the pairs of long stamens.

Silique. The fruit of Chinese cabbage is a glabrous silique, often called a pod. It is about 3-5 mm wide and sometimes over 7 cm long with two rows of seeds lying along the edges of the thin replum (false septum, an outgrowth of the placenta) which arose as a placental outgrowth (Fig. 4). A silique may contain from 10 to 25 seeds, depending upon the variety. About three to four weeks after the flower opens, the silique reaches its maximum length. When fully ripe and dry, dehiscence takes place through the two valves breaking away from below upwards, leaving the seeds attached to the placenta.

Seed. Seeds are usually globular to slightly oval in shape, about 1-2 mm in diameter, light brown at first, but becoming grayish black to red-brown later. Botanically, the seed is a mature fertilized ovule. After fertilization the endosperm develops immediately, although the embryo does not start growth for some days. The embryo is generally still small even after two weeks, but soon after it fills most of the seed as the endosperm becomes almost completely absorbed. The reserve food is stored in the cotyledons which are folded together with the radicle lying between them (conduplicate). The seed coat consists of the derivatives of two integuments. From outside to the interior, the following parts can be distinguished: a thin-walled and compressed epidermis, a layer of collapsed subepidermal tissue, a supporting layer of radially elongated cells with thickened, brown-colored sidewalls, and an irregular layer of pigmented cells. The seed coat is featureless although sometimes the radicle position is indicated by a low ridge.

Growth and Patterns of Head

The number of leaves of a fully grown Chinese cabbage plant range from 20 or more in the early-maturing variety to 100 or more in the late-maturing one. The differentiation of leaves is very rapid from the emergence stage to the initial heading stage (Fig. 5), and the inner leaf differentiation usually overlaps with the head development. Head development is strongly related with the leaf area of nonheading leaves (Kato 1981); the larger the nonheading leaf area, the bigger and more compact the head in the same genotype. The growth of head leaves, in terms of fresh weight, is very rapid after formation of head posture. The area of individual nonheading
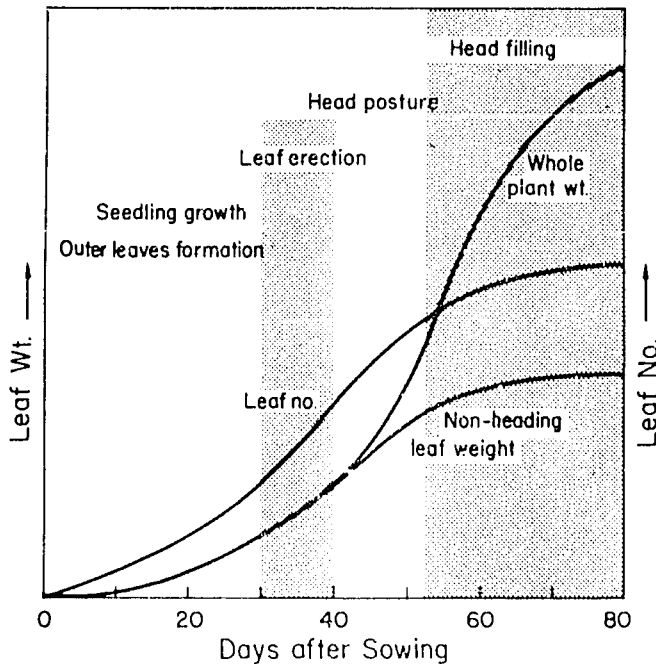
Figure 5.
Changes in leaf number and fresh weight during Chinese cabbage plant growth.

leaves increases with advancing leaf order, whereas the area of individual head leaves decreases from the outer head leaves to the innermost leaves. Head weight is positively correlated with the total number of leaves differentiated from the shoot apex and the growth of leaves after initial heading. The leaf area and leaf fresh weight of the head are also positively related with the leaf area and leaf fresh weight of nonheading leaves

The pattern of heading varies greatly with different varieties, and can be easily visualized if a cross section of a head is examined. Ito and Kato (1957) separated the organization or structure of leafy heads into: (1) the 'leaf weight' type, in which the heads are composed of fewer, rather heavy leaves; (2) the polyphyllous type, in which the heads are composed of many light leaves; and (3) the intermediate type.

The change in leaf shape during the development of Chinese cabbage, expressed by the ratio of leaf length to width (leaf shape index), is usually from oval to broad round, and successively from outer to inner leaves (Kato 1981). This usually occurs in the round and flattened types but not in the cylindrical types, where no such remarkable change in leaf shape index exists (Lee 1984). The change of leaf shape seems to be only a morphological characteristic and has no relation to head formation (Lee 1986).

Environmental Conditions for Vegetative Growth and Heading

Chinese cabbage grows best under cool conditions; it requires average temperatures of 22 °C for seedling emergence and formation of nonheading rosette leaves, and 16° to 20°C for head formation (Jiang 1981, Kato 1981). High day and low night temperatures are favorable for the heading process. The temperatures at which head formation occurs vary with varieties. The heat-tolerant, early-maturing varieties are able to head at mean temperatures relatively higher than 25°C (Opeña and Lo 1981).

High temperatures promote the production of narrow leaves, reduce the leaf growth, delay the initiation of heading, and increase petiole to blade ratio. Temperatures higher than 25°C prevent head posture formation (Kuo and Tsay 1981), enhance tipburn which is related with

calcium deficiency (Kuo et al. 1981b), increase respiratory loss of photosynthates and transpiration rate, resulting in poor head growth, and favor disease development (Lee 1984).

Light intensity also affects leaf growth and heading. High light intensity promotes the development of broad leaves and heading, whereas low light intensity encourages the growth of narrow leaves. Reduced light intensity causes a downward movement of the outer heading leaves. The head yield decreases with the light intensity lower than 200 cal./cm²/day. Day length does not affect head formation but may reduce the growth rate and weight of leaves (Kato 1981, Ootake 1979).

Chinese cabbage thrives well in a fertile, clayey loam soil because it requires a considerable amount of nutrients to sustain rapid growth in a short time. A large amount of nitrogen is required for the growth of the outer nonheading leaves and the differentiation of inner head leaves. The critical nitrogen content, below which deficiency symptoms develop, is about 1.5%; a nitrogen content of 2% to 3% is considered necessary for the normal head development and the inner leaf differentiation. The requirement for calcium is comparable to that for nitrogen. The function of calcium is particularly important in the head development because the head leaves, which embrace a growing point, can be seriously affected by calcium deficiency (Hara 1982).

The actual depth of the root system is affected by soil type, preplant cultivation, moisture supply, method of irrigation, oxygen supply, and adequacy of drainage. A strong root system helps avoid problems associated with drought stress later in the season. The majority of the root system of most transplants occupies 35 cm of the soil depth; therefore, the top 35 cm of soil must be irrigated regularly, especially during the dry season. A total of more than 25 l of water per plant is transpired from sowing to harvesting for tropical varieties. Since more than 90% of the plant's fresh weight is water, adequate irrigation to maintain soil moisture level between 65% and 85% of field capacity is extremely important for high yield. The water requirement greatly increases with advancing growth stages particularly during the heading stage (Kuo et al. 1988); drought stress in the heading stage prevents head formation (Kuo and Tsay 1981). Flooding and poor soil aeration during the rainy season are also detrimental to vegetative growth and head formation; plants usually die off within three days of flooding in the tropics because of the synergistic effect of high temperatures. Chinese cabbage is also sensitive to salinity stress (Shimose and Kurosaka 1985). The threshold level of salinity stress is about 1.5 dS/m, and of sodium ion about 20 meq l.

Physiology of Flowering

Flowering marks the transition from vegetative to reproductive stages in seed plants. It is, thus, a crucial event in the life cycle of plants, particularly from the standpoint of seed production. Flowers are modified shoots produced by the modified shoot meristems, the flower primordia. Once a meristem has been determined to be a flower primordium, it is usually unable to revert to vegetative growth. The central problem of the physiology of flowering is to understand which factors cause a shoot meristem to become a flower primordium, and how they consummate their action. It is important to note that flowering of all the *Brassica* vegetables is associated with 'bolting', the rapid elongation of the axis from the almost stemless whorls of leaves that is peculiar to the vegetative stage. Bolting is an easy indicator of flowering; however, it may not be equal to flowering. There are cases of stem elongation without flowering (Suge 1984).

The physiological control of flowering may be exerted at any of several fairly definitive development stages of the plant. Environmental cues may provoke the induction of the reproductive state — the initiation of floral meristems, the morphological development of flowers, and anthesis itself.

Reproductive development of the *Brassica* plant is usually triggered by such environmental variables as temperature and photoperiod (Friend 1985); low temperature and long-day conditions promote development of flower stalk (bolting) with or without flower formation in most species of *Brassica* (Kagawa 1971). The reproductive stage in *B. campestris* is usually set within three days after completion of triggering by these environmental variables (Orr 1978). Since these

environmental variables change with season, a programming of the reproductive activities on a seasonal basis or climatic conditions in a specific location is possible for Chinese cabbage (Matsui et al. 1981).

There is no definite sequence or relationship between head formation and floral bud differentiation. Floral buds could differentiate after or before the onset of heading (Fig. 6). This phenomenon occurs frequently when Chinese cabbage is grown as an autumn crop in the temperate region. If floral buds differentiate prior to heading, loose and unmarketable heads are formed.
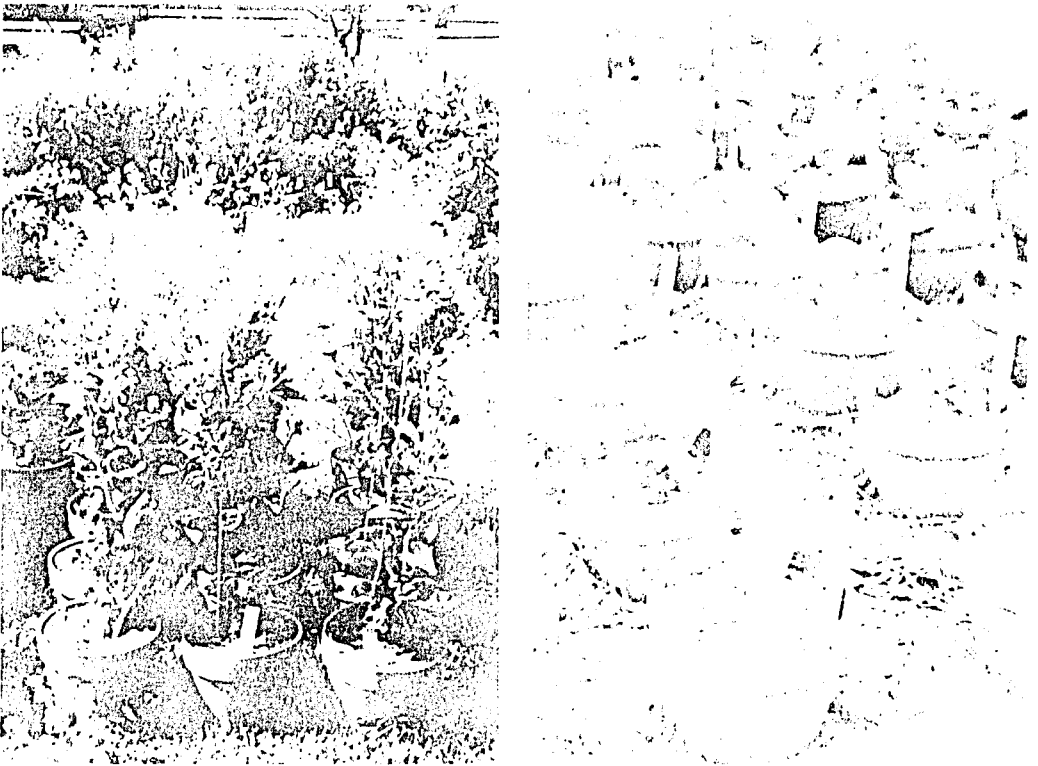
Figure 6. Flowering plants after vernalization at heading stage (left) and seed stage (right).

Vernalization. Many *Brassica* species are induced to flower by low temperature. The promotive effect of low temperature on flowering is termed 'vernalization'. The effects of low temperature can be observed in many subspecies of *B. campestris*, including subsp. *pekinensis*, when moistened seeds or any ensuing growth stages are chilled; in *B. oleracea* and its related subspecies, the effects can be obtained only when growing plants are chilled (Kagawa 1971, Mero and Honma 1984, Shinohara 1959). The above species are considered as seed vernalization and green-plant vernalization types, respectively. *B. nigra*, classified as a weak seed vernalization type, responds readily to chilling at the seedling stage. All amphidiploid species of *Brassica*, *B. napus*, *B. juncea*, and *B. carinata*, tend to have chilling response characteristics that are intermediate between their elementary parent species (Amagasa et al. 1987, Inouye and Kuo 1981, Kagawa 1971, Shinohara 1959).

The actual temperature greatly alters the effectiveness of any vernalization treatment; for most *Brassica* species, temperatures ranging from 0 to 5°C are optimal for artificial vernalization (Friend 1985). The longer the duration of chilling, the faster is the flower development. The temperature favorable for chilling for both *B. campestris* and *B. juncea* is not very low (Lee

and Sheo 1957). There are no appreciable differences in flowering of these two species when vernalized at the temperature range of 0° to 10°C. Some Chinese cabbage varieties may even produce flowers at 13°C or higher (Guttormsen and Moe 1985a and b). In other species, e.g. *B. oleracea*, the effective vernalization temperatures are sharply confined to the 0° to 5°C range. Flower induction in some subspecies of *B. oleracea* is extremely difficult and requires a longer vernalization period. At least six weeks of vernalization at 3°C of plants with at least 15 green leaves are necessary to bring about normal flowering of different subspecies of *B. oleraceae* (Marrewijk 1976, Stokes and Verkerk 1951, Thomas 1980). However, some tropical types of Chinese kale (*B. oleracea* subsp. *alboglabra*) may produce flowers without exposure to low temperatures (Lee and Sheo 1957, Shinohara 1959).

After vernalization, warm temperatures can have a depressing effect on the earliness of flowering. Temperatures above 26° to 30°C are known to retard flower development or reverse the vernalization effect on *B. campestris* subsp. *pekinensis* and *B. juncea* (Elers and Wiebe 1984b, Lee and Sheo 1957, Shin et al. 1987).
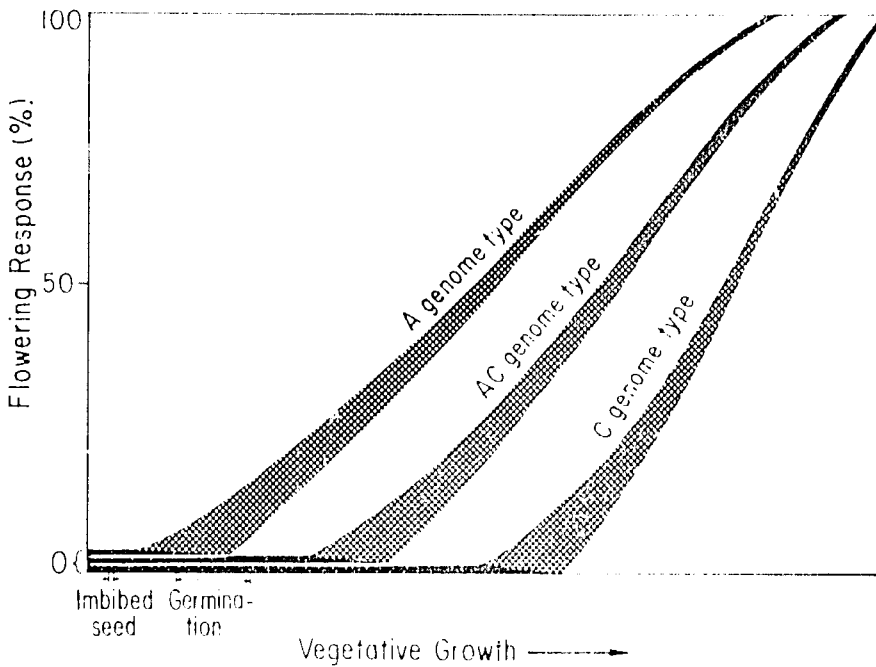
Figure 7. Relationship between the growth stage and the sensitivity of A, AC, and C genome types of *Brassica* to vernalization.

The sensitivity of Chinese cabbage to vernalization starts at germination (Elers and Wiebe 1984a, Guttormsen and Moe 1985a, Kagawa 1971), and increases with increasing plant age (Fig. 7) (Elers and Wiebe 1984a, Eguchi et al. 1963). During their development on mother plants, seeds do not respond to vernalization (Elers and Wiebe 1984a). Varietal differences also play an important role (Guttormsen and Moe 1985a, Honma 1981). Heat-tolerant varieties tend to be more sensitive to vernalization (Guttormsen and Moe 1985a); this principle has been exploited as the screening technique for heat tolerance in segregating population (AVRDC 1975). Flower induction of Chinese cabbage is hastened as the temperature is lowered (Lorenz 1946); temperatures above 18°C are required to avoid premature bolting (Guttormsen and Moe 1985b). The critical temperature for vernalization lies below 13°C (Yamasaki 1956), with the optimum between 5° to 8°C (Elers and Wiebe 1984a). The number of days from seeding to flowering

of Chinese cabbage decreases with increasing duration of vernalization (Lee and Sheo 1957). For slight vernalization effects, one week is sufficient; for complete vernalization, about three weeks or more are needed (Elers and Wiebe 1984a, Lee and Sheo 1957). To achieve the same result, increasing the vernalization period is more effective than lowering the vernalization temperature (Elers and Wiebe 1984a, Matsui 1981). Exposures to temperatures above 16°C after the completion of vernalization delay the flowering, usually bud formation, of Chinese cabbage (Elers and Wiebe 1984b). High temperature treatments to the root system also have similar effects (Pressman and Negbi 1981).

Photoperiod. Most *Brassica* species are considered to be quantitatively long day plants (Friend 1985). The longer the daylength under which the plant is grown, the more extensive and earlier the flowering becomes (Elers and Wiebe 1984a and b, Kagawa 1971, Lee and Sheo 1957, Matsui et al. 1981, Zee 1975). The minimum period of long day cycles is one day for the oil seed type of *B. campestris* (Friend 1985), six days for *B. campestris* ssp. *parachinensis* or Chinese flowering cabbage (Zee 1975), and at least nine days for heat-tolerant Chinese cabbage (AVRDC 1977). The older the plant before photoinduction, the greater is the flowering percentage obtained at a given daylength.

Many *Brassica* species which respond to vernalization are also sensitive to long day stimulation of flowering. The interaction of these two factors are either supplementary or complementary. Thus, low temperature may quantitatively displace the long day requirement. Matsui et al. (1981) indicated that the effect of daylength on flowering of *B. campestris* subsp. *pekinensis* is greater than that of chilling temperature and is smaller than that of chilling duration. Long day promotes bolting when temperatures rise after incomplete vernalization of Chinese cabbage (Elers and Wiebe 1984a). Some sensitive cultivars can bolt and flower without experiencing temperatures below 15°C, if grown continuously under long day conditions (Lorenz 1946, Suge 1984, Suge and Takahashi 1982). Kagawa (1971) also showed that the dominant grading order of the hereditary nature of flowering is photoinduction > seed vernalization > green plant vernalization. However, it is evident that daylength is a more effective signal in the middle latitudes than in the tropics and more common there as a physiological cue to *Brassica* species.

It appears that there is no critical daylength (or it is shorter than eight hours if it exists) for *Brassica* species (Friend 1985, Zee 1975), but a combination of vernalization and long day is required for the maximum flowering of Chinese cabbage (Lorenz 1946). There are only slight photoperiodic fluctuations around 12 hours and a small temperature flux in the tropics. Thus, the increasing suitability of temperatures with rising altitude should play a more important role in the control of flowering in *Brassica* species in the tropics.

Regulation of flowering by growth substances. Growth substances, or hormones, have been found to play important roles in the control of flowering. Of all plant hormones, gibberellins (GAs) are the most effective. GA application replaces the requirement for long day or low temperature in some *Brassica* species (Lang 1965) and accelerates flowering of Chinese cabbage when it is applied during seed vernalization or vegetative growth (Kagawa 1966). Endogenous GA content of vernalized Chinese cabbage seedlings also increases within the first few days under long-day conditions (Suge and Takahashi 1982). However, not all cold-requiring or long day requiring *Brassica* species can be induced to flower with GA (Kahangi and Waithaka 1981, Suge 1984). There are cases in which GAs cause stem elongation without flowering on some difficult-to-flower *Brassica* species (Amagasa et al. 1987). Exogenous application of GA, without vernalization, induced flowering in easy to flower cultivars of *B. campestris* subsp. *pekinensis*, but not in difficult-to-flower cultivars, and raised the percentage of plants that flowered under short days (Kagawa 1966, Suge 1984, Suge and Takahashi 1982). GAs seem to play a role in the bolting of Chinese cabbage plants, but probably are not directly functional in initiating flowering (Suge and Takahashi 1982).

A combination of vernalization with GA application was proposed as an alternative to long-day treatment or incomplete vernalization in order to bring about flowering in some difficult-to-

flower *B. oleracea* and *B. napus* (Ali and Machado 1982, van Marrewijk 1976). It was suggested that seed production and breeding programs on *Brassica* may be enhanced by a combination of vernalization and GA, thereby, saving substantial time and energy (Ali and Machado 1982). The combination of vernalization and GA application is appealing for the practical purpose of seed production; however, the effectiveness of the method depends on the crop variety. Most heat-tolerant Chinese cabbage varieties tend to flower easily (AVRDC 1975); therefore, the above method may be applicable.

Flowering.   The optimum temperatures for flowering range from 18° to 25°C. Temperatures above 32°C usually result in abnormal floral development with enlarged sepal but defective anthers (Jiang 1981), and poor pollen production and viability (Kuo et al. 1981a), resulting in poor or no silique setting. The optimum relative humidity (RH) for anthesis is 60-70%; RH above 90% is not favorable for the flowering and pollination process.

Silique and Seed Development

With the successful fertilization of the flower, a burst of growth of the erstwhile ovary and ovule occurs, and the development of the silique and seed begins, usually with a simultaneous wilting and abscission of the petals and of the stamens. Investigations into the silique and seed development of *Brassica* vegetables have been scarce.

The seed and silique development of *B. napus* may be divided into three stages (Norton and Harris 1975). In the first stage, while the plants are still flowering, silique wall development is rapid but seed growth is slow. In the second stage, silique wall growth continues to be rapid but this period marks the onset of embryo development and the deposition of seed storage materials. During this phase there is a rapid increase in seed dry weight and marked changes in composition. In the third stage, seed weight more than doubles while the gross chemical composition remains almost in constant proportion. Seed development in *B. napus* appears to be complete in about five weeks with only dehydration occurring in the remaining two weeks. In this period the silique wall exports considerable dry matter to the developing seed before it matures. Seeds of Chinese cabbage mature faster than seeds of *B. juncea*. Under favorable conditions it takes only five to six weeks from flowering to full seed maturity in most Chinese cabbages.

The number of siliques that develop in the *Brassica* genus usually remains constant regardless of environmental conditions (Kuo et al. 1981a, Norton and Harris 1975), indicating that losses due to abscission are minimal. However, the seed number within a silique usually decreases during the silique development even under favorable conditions (Norton and Harris 1975). The decrease is due to the rapid failure of embryo development, followed by a gradual decline in seed development, and finally the exclusion of poorly developed or damaged seeds at maturity. The reason for this decline is obscure, but competition for assimilates may be responsible and bear a close relationship to environmental conditions.

Both pre- and postflowering growth have great influence on the seed yield of *Brassica* species (Brar and Thies 1977, Thurling 1974). Seed yields decrease with a reduced period of vegetative development; they increase with an increased dry matter accumulation in the period between anthesis and final harvest (Thurling 1974, Thurling and Das 1980). It was estimated that the contribution to the dry matter accumulation in the seeds was 37% from the leaves, 32% from the silique walls, and 31% from the stem. Nearly 75% of the assimilates from the topmost leaf were translocated to the growing siliques (Brar and Thies 1977). The siliques have a photosynthetic function and provide a considerable amount of photosynthates to the developing seed (Hozyo et al. 1972).

The optimal temperature for seed setting and development is around 17°C; exposure of developing siliques to 32°C usually leads to empty siliques in *B. campestris* subsp. *pekinensis* (Inomata 1976). The reduction of seed number by high temperature may be possible because it affects both male and female gametogenesis (Kuo et al. 1981a). Furthermore, *in vitro* pollen

viability tests indicate that the optimum temperature for germination and growth of pollen in *B. campestris* subsp. *pekinensis* is around 20°C (Kuo et al. 1981a). The seed-setting ability of bud-pollinated Chinese cabbage flowers is also closely related to atmospheric temperatures (Tao et al. 1982). Thus, high temperature is a major factor limiting good seed production of Chinese cabbage in the tropics because it not only retards flowering but also seed setting and development.

The optimal RH for seed setting and development is from 50% to 60%; RH higher than 80% reduces photosynthesis, thus seed development.

Seed Dormancy and Germination

Seeds of *Brassica* vegetables exhibit dormancy for a certain period after harvest. The dormancy period varies with species and cultivars, normally ranging from 0 to 140 days (Watanabe 1953). For *B. campestris* subsp. *pekinensis* and subsp. *rapa*, dormancy disappears rapidly and the period is usually short; for *B. oleracea*, dormancy ranges from short to long; and for *B. napus* and *B. juncea*, dormancy is usually long. Seeds of *B. napus* and *B. cernua* show a prolonged dormancy period of two years or more when they are preserved in the harvested siliques (Tokumasu 1975). The removal of dormancy is usually delayed when *Brassica* seeds are stored under extremely dry or humid conditions. The optimal RH range for the removal of seed dormancy in *Brassica* species is from 10% to 70%, depending upon species and varieties (Tokumasu et al. 1975 and 1981a and b). Dormancy of most *Brassica* vegetables can be broken by germinating the seeds on a filter paper impregnated with 100 ppm gibberellin A_3 (Watanabe 1959).

The mean germination period is shortest and the percentage of germination highest from 25° to 35°C for Chinese cabbage and other *Brassica* vegetables (Tokumasu et al. 1985). However, the response of dormant seeds is different from nondormant seeds; the optimum temperatures are usually low (15° to 25°C) for dormant seeds. There are also varietal differences in the germination response to temperature (Kondra et al. 1983).

Breeding System and Natural Mechanisms for Hybridity

Population Structure

Chinese cabbage is normally insect pollinated, commonly by honeybees, and therefore, practices an allogamous (outcrossing) type of mating. The sticky pollen is not wind-blown. An open-pollinated cultivar of this crop can be considered as made up of freely interbreeding individuals sharing a common gene pool.

As with other outcrossing plant species, individuals in an open pollinated variety of Chinese cabbage genetically differ from each other, are highly heterozygous, and exhibit depressed vigor upon enforced inbreeding. Heterozygosity is an essential feature of this crop and, as a result, this genetic condition must either be maintained during the breeding process or, as in the case of hybrid breeding, dissipated during the development of inbred parents but restored in the final step.

Experienced Chinese cabbage breeders generally agree that genetic differences exist in the expression of inbreeding depression. One type shows a rapid decline of vigor in the initial four to five generations of selfing; another maintains 70% to 80% of the original vigor until the 7th to 8th selfing generation; and still another shows an initial decrease of vigor for a few generations, but thereafter the lines somewhat recover and stabilize in vigor (Fujii 1972). In the course of obtaining the desired genetic purity, it is important to identify those lines which exhibit the least inbreeding depression.

Genetical and evolutionary studies in recent years have provided a formidable body of evidence indicating that heterozygosity is intimately connected with the efficient functioning of an outcrossing population. The implication is that, in order to maintain high fitness, outcrossing species cannot afford to dispense with heterozygosity, and thus, they must have the means to encourage or enforce cross-pollination. Of several known hybridity mechanisms that many outcrossing species have adopted to encourage or enforce cross pollination, and thus, preserve heterozygosity, such as monoecy, dioecy, protandry, protogyny, and incompatibility, self-incompatibility is the regular system for cruciferous plants.

Self-incompatibility is a system of mating control which enforces nearly complete, if not *en toto*, outcrossing in many species which have adopted it. Along with dioecy, which enforces complete outcrossing, these powerful systems are genetically controlled, and therefore, genetic implications arise when artificial manipulation of pollination, such as in a breeding program, is carried out. Self-incompatibility (SI) is the regular system for cruciferous plants.

Self-incompatibility

Physiology of self-incompatibility. Self-incompatibility refers to the partial or complete inability of a fertile pollen to set a viable seed after self-pollination. It is widely distributed among the flowering plants (Heslop-Harrison 1983) and can be classified into two types, though the flowering structures are similar in both the pollen and seed parents (i.e. homomorphic) (Fig. 8):

1. Gametophytic self-incompatibility. The pollen/pistil interaction is genetically controlled by the haploid genome of each pollen grain and the diploid genome of the pistil tissue. The hindrance to pollen tube growth is in the style. Genetically, a single locus with many alleles is involved. Pollen grain is generally binucleate.
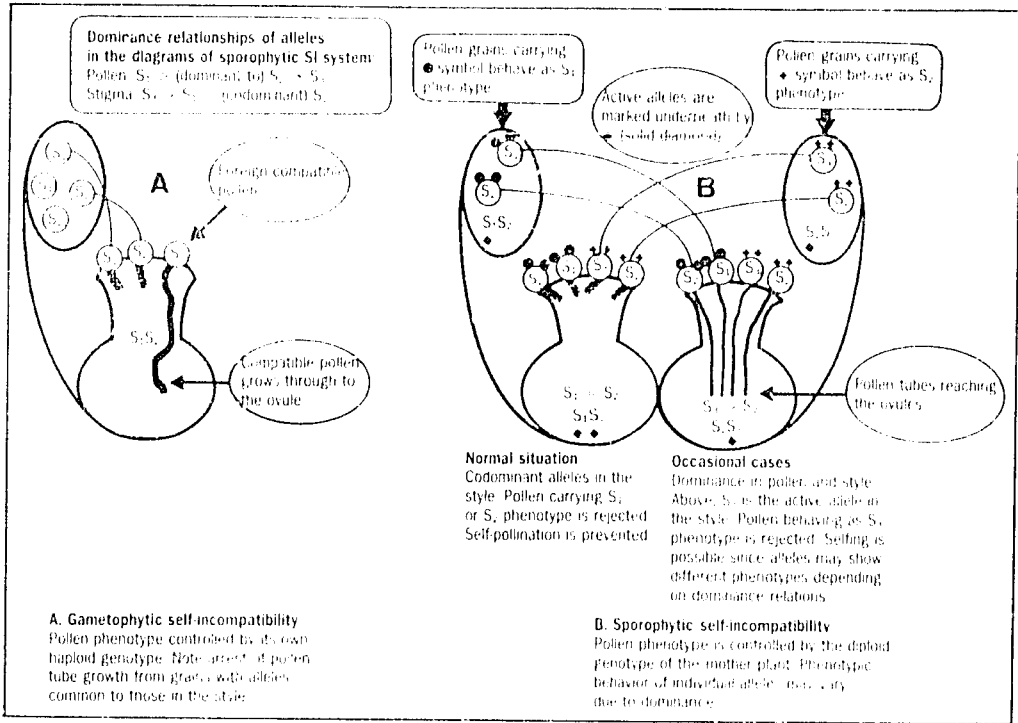
Figure 8.   Comparison between multiallelic gametophytic incompatibility and sporophytic incompatibility.

2. Sporophytic self-incompatibility. The interaction of the pollen and ovule is determined by the genome of the diploid somatic tissue (of the sporophyte) in which the pollen and the ovule are developed. In this system hindrance to pollen germination or pollen tube growth is localized in the surface of the stigma. Genetic control of this system is also by a single locus with many alleles exhibiting allelic interaction. Pollen grain is generally trinucleate. This system is typical among crucifers.

Anatomically, the stigma of cruciferous plants with a sporophytic SI system is covered with a layer of papilla cells on its surface and belongs to the so-called 'dry stigma'. The cell wall of papilla cells is composed of an inner pectin-cellulose layer and an outer cuticle, on which waxes are deposited (Kroh 1964). Compatible pollination allows the pollen tubes to pierce the cuticle layer and grow through the pectin-cellulose layer toward the conducting tissues, dissolving the pectin in the process (Kroh 1964). In incompatible pollinations penetration into the cuticle layer occurs to a certain degree but penetrating tubes cannot grow through the pectin-cellulose layer (Dickinson and Lewis 1973, Kanno and Hinata 1969). Self-incompatibility in crucifers is believed to be the result of interactions between stigmatic papilla cells and pollen or pollen tubes since self-pollination after stigma mutilation can yield selfed seeds (Tatebe 1939)

Stages of pollen germination and tube development: A stigma is capable of receiving both self and nonself pollens, and ovules are potentially fertile. However, various possible interactions take place from pollination to fertilization in an efficient SI system. Ferrari et al. (1981) identified five distinct stages for *Brassica* pollen germination and pollen tube development (Fig. 9). The first four stages are autotrophic and are programmed within the mature pollen grain to occur before the pollen tube penetrates into the female tissues. Except for self-recognition, these stages do not require specific messages from the female tissues. Pollen tube development can be arrested at these stages, depending on environmental conditions other than pollen-pistil interaction. These stages are listed as follows:
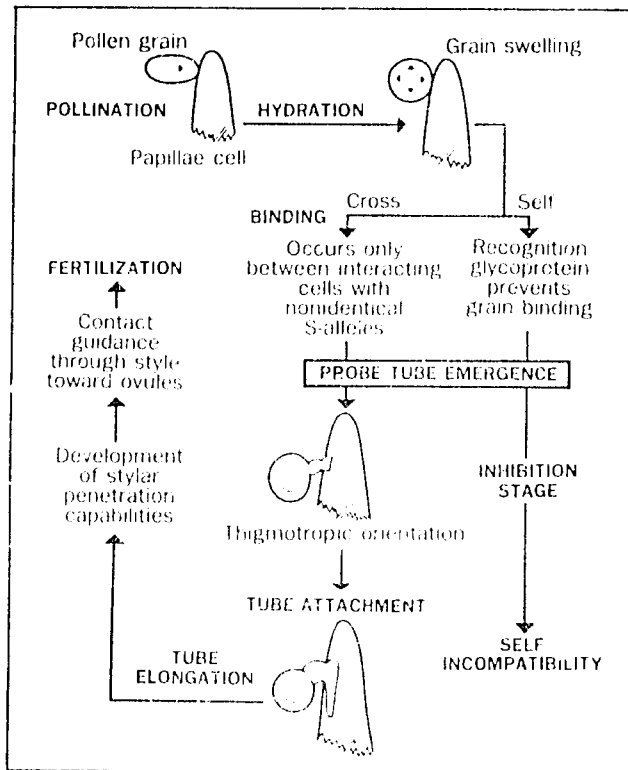
Figure 9.
Stages of pollen germination and pollen tube development in *Brassica* (from Ferrari et al. 1981).

1. Primary binding stage. The first postpollination stage of pollen development is a primary binding of the grain to the stigma papillae. Binding is greater for cross-pollination than self-pollination. This primary binding is a loose, sticky adhesion of the pollen to the papillae, derived from the proteinaceous components of the stigma pellicle and pollen wall.

2. Hydration stage. The second stage involves water uptake, which begins at RH higher than 50%. Following hydration, cell turgor at a higher RH is essential for germ tube formation. A hydrophilic factor of low molecular weight in the stigma is responsible for reducing 'hydraulic resistance' in parts of the 'water pathway' leading from the stigma cells to the pollen grain, and increasing the RH at the stigma surface by attracting water vapor.

3. Probe tube stage. A short probe tube emerges from most pollen grains, attaining a maximum length of about one grain diameter on a substratum. All probe tubes originate at and develop toward the substratum interface.

4. Probe tube attachment stage. The pollen tube attachment develops if the probe tube strikes a compatible stigma. When attachment does not occur tube elongation ceases at the probe stage and callose deposition fills the probe tube. In the successful tube attachment, the transfer of nutritive precursors from the female tissues takes place, permitting the continuing growth of the pollen tube.

5. Pollen tube elongation. The penetration of pollen tube, which frequently attains a length of more than 100 grain diameter, into the compatible stigma and then the stylar tissues, is activated by the stylar tissue.

Self-recognition and control of incompatibility response: Pollen tube development beyond the probe tube stage is prevented by the consequence of self-recognition. The self-recognition event involves a genotype-specific interaction of a mobile informational molecule on the stigma papillae, with a receptor molecule permanently located in or on the pollen grain. This recognition reaction is a prerequisite to the manifestation of self-incompatibility.

In incompatible pollination, probe tube growth ceases after about three hours on an incompatible stigma, whereupon the primary probe tube becomes occluded with callose. It has been suggested that an endogenous, probe tube-localized inhibitory mechanism communicates with the pollen grain so that binding and tube attachment cannot occur, thus blocking the continued tube development. That, in turn, causes callose production.

The pollen grain: It is now known that chemical fractions carried in the pollen wall do play a part in incompatibility response. According to Heslop-Harrison (1975 and 1983), *S*-gene products (proteinaceous substances) accumulating in the innermost layer of the pollen sac wall are transferred, upon dissolution of the tissue, to the outer wall of the mature pollen grain during the final stages of pollen maturation, about 60-70 hours before anther dehiscence in crucifers. Soon after landing on the stigma, the pollen releases these proteinaceous substances within minutes. When these substances come in contact with the incompatible stigma papilla, they cause immediate callose (β-1,3-glucan) production on the stigma papilla. The penetration of the papilla wall by the pollen tube, an essential first step in its successful progress into the style, is then prevented. This protein-induced callose production does not occur when the pollen source is compatible with the stigma.

The pollen-stigma reaction is actually a cell-to-cell reaction; the callose production is restricted to the very same papillae which come in contact with the incompatible pollen grain. Thus far, attempts to detect differences in the pollen proteins of wall diffusates or whole pollen extracts in sporophytic self-incompatible *Brassica* species by immuno-electrophoresis have been unsuccessful (Ferrari et al. 1981, Heslop-Harrison 1983).

The stigma: The relatively 'dry' stigmatic surface of cruciferous plants, being peculiarly covered with a hydrated overlayer of a proteinaceous pellicle on the surface of the papilla cuticle, is responsible for the localization of the pollen-stigma interaction. The stigma surface pellicle in this way forms a receptor site for the sporophytic pollen wall proteins.

In the course of pistil maturation, the papilla cells in self-incompatible strains stop elongating two or three days before anthesis. This retardation of papilla growth seems to coincide with the acquisition of their discriminating ability (Gonai and Hinata 1971), and has been suggested to activate the proteins responsible for the recognition reaction (Hinata and Nishio 1980).

Serologically detectable macromolecules from stigmas have been correlated with *S*-alleles (Hinata et al. 1982). Very low quantities of this molecule are present on stigmas of immature flower buds, where self-pollination functions normally. The quantity increases as buds develop into mature flowers; this being accompanied by increased expression of incompatibility. This molecule was named the *S*-specific protein or *S*-glycoprotein because the protein contains carbohydrates. In contrast with the results from pollen, striking differences in the patterns of stigma antigens (*S*-glycoproteins) related to incompatibility genotype exist in *Brassica* species; these antigens are heritable and correlate with *S*-alleles segregation (Hinata 1981). The relative mobility of stigmatic *S*-glycoprotein bands on the immuno-electrophoresis has been used as a source of information to predict the identity of *S*-alleles (Nishio and Hinata 1980). Thus far, eight *S*-glycoproteins have been identified with their respective *S*-alleles in *B. oleracea*, and four in *B. campestris*. Evidence suggests that although the *S*-gene-specific antigens are present in considerable amounts in the stigmatic tissue, they are not present in the stylar nor anther tissue of the flower, and that the *S*-gene-related fractions are on or very near the surfaces of the stigma papillae (Nasrallah and Nasrallah 1984). The possibility is obvious that they are species of proteins present in the pellicle, and are responsible for the recognition reaction.

Furthermore, low molecular weight compounds, which are responsible for the subsequent inhibition of pollen germination and pollen tube growth, have been detected in the stigma of *Brassica oleracea* (Hodgkin and Lyon 1984). Some of these inhibitors, as examined by thin-layered chromatographic bioassay, are detected only in stigma extracts following self-incompatible self-pollinations. Inhibitors from self-pollinated stigmas are at their highest concentration about two hours after pollination, indicating there is a rapid accumulation of the inhibitors (Hodgkin

and Lyon 1986). These inhibitors may play a part in preventing the germination and growth of *Brassica* pollen in incompatible pollinations.

Modifications of self-incompatibility expression.

Modifications of the breeding behavior of self-incompatible plants may result, spontaneously or after experimental manipulation, from either physiological or genetical changes. These changes interfere, at one or several stages of the rejection process, to alter the chain of events leading to the failure of fertilization process after self-pollination. The physiological changes are always temporary and cannot be transmitted to the next generation. Their effects, when they contribute to the promotion of inbreeding or to variation in mating relationships, may have important implications for the genetic structure and the fitness of the population in which they occur.

Genetical changes, on the other hand, may be permanent and cover a variety of different effects ranging from the breakdown of the incompatibility character to an increase in the size of *S*-allele series, or to the emergence of a new relationship between pollen and pistil. For example, self-incompatibility in Chinese cabbage needs at least three or more generations to be genetically stabilized and the stability differs with different varieties (Anonymous 1976, Tao et al. 1982). This section summarizes the most important environmental and physiological factors affecting self-incompatibility.

Stage of pistil development:

Seed set in self- and cross-pollinations changes during the course of pistil growth. Very young pistils are too immature to set seeds. As buds grow, both self- and cross-pollinations with mature pollen yield good seed set. This is known as 'bud pollination', and the bud pistils do not discriminate between the genotypes of self and nonself. Usually seed set from selfing is poor around five days before and after the opening of flowers, while seed set from cross-pollination remains good (Fig. 10). The difference in seed set between compatible (cross) and incompatible (self) pollinations is at its highest from the day before to the day after flower opening.
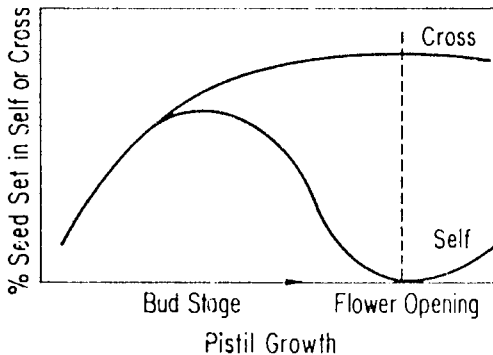
Figure 10.
Schematic presentation of the changes of seed set percentage in self- and cross-pollination in the course of pistil growth.

The difference of compatible/incompatible reactions in pistil development can be readily distinguished by the increased size and the appearance of yellow pigments in the self-incompatible buds.

The success of bud pollination is attributable to bud pistils not having the self- and nonself-recognition ability present among blooming flowers (Iizuka 1957). Apparently, the bud has not yet received the necessary information for inhibiting self-pollen (harvested from mature flowers).

The increasing expression of self-incompatibility with the advanced pistil growth is closely associated with the growth retardation of papilla cells near anthesis (Gonai 1970, Gonai and Hinata 1971). Mature papilla cells disturb the penetration of pollen tubes but not bud papilla cells in self-incompatible *Brassicas* species (Ockendon 1972). Furthermore, *S*-proteins detected

by the immunological method (Nasrallah 1974) and S-glycoproteins detected by isoelectric focusing (Hinata et al. 1982, Nishio and Hinata 1977) increase with the course of stigma maturation, which is correlated with the onset of the incompatibility response (Nasrallah and Nasrallah 1984, Shivanna et al. 1978). The proteins involved in the recognition of self-incompatibility are not secreted in the papilla cells at the bud stage. The acceptable hypothesis, therefore, is that S-alleles in papilla cells are activated and that the substances involved in self-/nonself-recognition are produced during papilla growth retardation.

Young pistils virtually do not differ in their ability to set seeds upon selfing and crossing. This 'juvenile compatibility' upon selfing has been noticed as early as 1930 by Kakizaki, and selfing by 'bud pollination' was subsequently developed as a routine technique to obtain selfed seeds. The implication of this phenomenon in the maintenance of S-allele homozygous inbred lines for F_1 hybrid production is discussed later (see Hybrid Seed Production, p. 61).

Aged pistils, those that remain unpollinated a few days after flower opening, can still produce a substantial amount of cross seeds and sometimes, may set a few seeds upon self-pollination with normal incompatible pollens. The ability of aged flowers to set selfed seeds, known as 'senile compatibility', is associated with a progressive loss of the capacity to produce or store active incompatibility substances or the collapsing of papilla cells (Tatebe 1977).

Temperature: Higher rate of selfed-seed production occurs at high temperatures (25° to 30°C) than at low temperatures (15° to 20°C) in *B. oleracea* (Gonai and Hinata 1971, Johnson 1971, Nasrallah and Wallace 1968, Oekendon 1973, Visser 1977), in *B. campestris* (Ko et al. 1974, 1975, 1976, Richards and Thurling 1973), and in *R. sativus* (El Murabaa 1957, Tatebe 1977). Thermal treatment (70° to 80°C) with a small soldering iron applied to the stigmatic papillae also markedly increased fertility in self-incompatible crucifers (Roggen and van Dijk 1976).

A higher proportion of collapsing papilla cells took place under high (30°C) than low (20°C) temperatures and was considered as the main cause of the high selfing rate at high temperatures (Ko et al. 1976, Tatebe 1977). Other postulated reasons for the influence of high temperature on degree of self-incompatibility are: changes in the rate of synthesis or quality of synthesized incompatibility substances (Nasrallah and Wallace 1968); and shortening of the papilla growth retardation at high temperature, thus disturbing the full expression of self-incompatibility (Gonai and Hinata 1971).

The effect of high temperature on self-incompatibility has also been observed to vary among different genotypes and different S-alleles. For example, the expression of self-incompatibility among the progenies of self-incompatible lines in *B. campestris* is primarily determined by the interaction between S-genotypes and different combinations of polygenic modifiers, which are temperature-sensitive and probably differ from one another in their temperature requirements (Richards and Thurling 1973). Although rather difficult, it is possible, therefore, to find S-alleles which are stable under high temperatures.

Relative humidity: High air humidity has been found to increase pollen germination in *Brassica* and *Raphanus* (Oelke 1957, Robbelen 1960, Tatebe 1964), and accelerate pollen tube growth in both self- and cross-pollinations in *B. oleracea* (Kanno 1973). At the same relative humidity, the rate of pollen germination was less in self- than in cross-pollination. With strong self-incompatibility such as in *B. oleracea*, humidity did not improve self-fertility (van Marrewijk and Visser 1978). In contrast pollen tube penetration could be observed in weakly self-incompatible lines under high humidity.

Gas environment: A high concentration of CO_2, e.g. 3% to 5% can cause a breakdown of self-incompatibility in newly opened flowers of crucifers (Nakanishi et al. 1969, Nakanishi and Hinata 1973, 1975). The effectiveness of CO_2 is limited to the pollen germination period and the effect is considered to involve pollen tube penetration disturbances rather than the recognition phase. The reactions concerned and the exact nature of the CO_2 effect on pollen

tube penetration are not known, but it certainly appears that the processes involved in the incompatibility reaction are more sensitive to external agents than those normally governing pollen germina , pollen tube growth, and fertilization.

Lower ethy   ne concentrations appeared to be slightly antagonistic to enhanced pollen tube penetration caused by a high CO_2 concentration. Oxygen, on the other hand, did not have any effect in breaking self-incompatibility although it is necessary for metabolism in pollen germination and pollen tube penetration (Nakanishi 1972).

Chemical treatment: Considerable success in overcoming self-incompatibility of *Brassica* was obtained with cutinase (Linskens 1961), ether, or 10% KOH solution (Tatebe 1968), some organic solvents (Gonai and Hinata 1969), lectins or sugars (Bajaj and Shivanna 1986), and salt solution (Tao and Yang 1986). Self-fertility of inbred lines in *B. oleracea* with relatively weak self-incompatibility can also be increased by washing pollen with 10% to 50% acetone or by applying an ether extract from the pollen of rape to the stigma (Roggen 1974, 1975). The treatment of the pistil or of the pollen with chemicals seems to block recognition molecules and thus overcome self-incompatibility.

Mutilation: Mutilation of the stigma preceding self-pollination in incompatible broccoli (Sears 1937) and radish (Tabebe 1939) led to high seed set. Roggen and van Dijk (1972) proposed the use of steel wire for stigma mutilation to obtain high-selfing rate.

Irradiation: A significant increase in self-fertility was also obtained in self-incompatible cabbage by gamma irradiation applied 6-14 hours after pollination (Hosoda et al. 1971). The effect is likely due to an increase in the capacity of the incompatible pollen tube to bypass the incompatibility barrier and accomplish fertilization.

Electric-aided pollination: Electric-aided pollination (involves applying a direct electric potential difference of 100 V between pollen and stigma) could partially break self-incompatibility in *B. oleracea* and increase selfing rate in bud pollination (Roggen and van Dijk 1973). The effect was found to be of the same order of magnitude as that obtained by removal of the stigma, pollen transplantation, chemical treatment, temperature treatment, and bud pollination, and was more effective than CO_2 treatment (Ito 1981).

Genetical control of self-incompatibility. Kakizaki (1922) reported the first case of self-incompatibility in Chinese cabbage, and postulated that self-incompatibility in cabbage can be explained by the gametophytic action of two allelic series (Kakizaki 1930). This theory was later disproved on theoretical grounds by Bateman (1952, 1954, 1955) who proposed that self-incompatibility in crucifers is governed by the sporophytic action of one *S*-allelic series. Thereafter, the sporophytic *S*-allele system was found in *B. oleracea*, *B. campestris*, *Raphanus sativus*, and *R. raphanistrum* (Haruta 1962, Sampson 1957, 1964, Tatebe 1962, Thompson 1957, Zuberi et al. 1981). in Cruciferae, of 182 species examined, 80 were self-incompatible (Bateman 1955). In the subtribe Brassicinae which is comprised of *Brassica* crops and its closest wild allies, 50 out of 59 taxa examined were self-incompatible (Takahata and Hinata 1980). Self-incompatibility may have played an important role in the diversification of species in Cruciferae, especially in the subtribe Brassicinae (Hinata and Nishio 1980).

The genetic combinations of *S*-alleles are numerous and complex among crucifers with the sporophytic SI system. For example, 25 to 34 different *S*-alleles have been estimated in a population of *R. raphanistrum* (Sampson 1967); 41 have been identified in cultivated *B. oleracea* (Ockendon 1974, 1975b), and 10 in Chinese cabbage (Lee and Yoon 1981).

Evidence indicates that the cultivated forms of *Brassica* possess weaker self-incompatibility than do wild types (Ockendon 1974, Olsson 1960a and b, Thompson and Taylor 1966) and that the cultivated taxa have lost the dominant alleles to a certain extent. It could be considered that

certain factors, such as cultivation, have disturbed the genetic balance for self-incompatibility and made them self-compatible (Hinata and Nishio 1980). Interestingly, strong and stable self-incompatibility was infrequently observed among AVRDC's tropical Chinese cabbage germplasm.

In a simplified nomenclature for the sporophytic SI system, determined by a series of multiple alleles, the genetical constitution of individuals may be represented as S_1S_2, S_1S_3, S_1S_4, ...S_2S_3, S_2S_4, and so on. Assuming that the two S-alleles of a plant act independently of each other, the pollen of a plant deposited on the stigma of the same plant (selfing), or of another plant possessing one or both S-factors in common, e.g. $S_1S_2 \times S_1S_2$ or $S_1S_2 \times S_1S_3$, will produce very few seeds if any. Compatible crosses would only be those in which the parent plants possess no corresponding S-factors, e.g. $S_1S_2 \times S_3S_4$ or $S_1S_3 \times S_2S_4$. The phenomenon of cross-incompatibility is attributable to the same cause as self-incompatibility.

The above supposition that the two S-alleles act independently of each other occurs rarely in real situations, since dominance and codominance among S-alleles are a characteristic feature of the sporophytic SI system. As an example, if a plant has an S_1S_2 genotype and S_1 is dominant to S_2 in pollen, then all of the pollen from the plant will function as if it were S_1, and the pollen with either S_1 or S_2 alleles will be incompatible in an S_1 style, but will be compatible to an S_2S_2 style. In another instance, if S_4 is dominant to S_1 in pollen, the cross S_1S_2 (female) $\times$ S_1S_3 (male), which is incompatible on the assumption of independent S-allele action, would now be compatible.

Codominance among S-alleles is a relatively more common genetic phenomenon than dominance. Moreover, the relationship between S-alleles in the pollen is not always the same as in the stigma. By these conditions, as well as the fact that a large number of S-factors exist in a population, the number of compatible combination increases and cross-incompatibility is rather infrequent in natural open-pollinated populations but not in genetic materials that are composed of closely related individuals such as an inbred family. The possibility of obtaining individuals that are homozygous for the S-factors is rather obvious from the attribute of dominance or other pertinent combinations. Thus, in contrast to the gametophytic incompatibility system, homozygotes are also a normal part of the sporophytic incompatibility system (Lewis 1954). This feature has been utilized in the derivation of inbred lines that are homozygous for S-alleles, and has a very important implication in hybrid breeding.

Although S-alleles can be broadly classified into dominant and recessive S-alleles, complications generally arise because of their differential behavior in the pollen and stigma. Dominance relations are often nonlinear (Lee and Yoon 1981, Ockendon 1975a, Richards and Thurling 1973, Thompson and Taylor 1966). The degree of dominance can also change owing to genetic backgrounds, and physiological or environmental conditions (Murakami 1965, Ockendon 1975a). Competitive interactions can also induce recessive heterozygotes to change to self-compatibility (Lawson and Williams 1976a and b).

Self-compatible plants have been found occasionally in self-incompatible populations (Nieuwhof 1968a); seeds may set from pollen carrying the same allele that is present in the stylar tissue. This condition is referred to as pseudo-self-compatibility. The amount of pseudo-self-compatibility may be modified by environmental factors as described above, or perhaps by modifying genes. In addition it is assumed to have been due to the presence of an Sf gene of S-allelomorphs which renders ineffective the alleles for incompatibility (Bateman 1954, Nasrallah 1974, Thompson and Taylor 1971). The Sf allele is a part of the S-allele series and may arise by mutation from an S-allele. The existence of polygene systems which modify the expression of self-incompatibility has been pointed out (Haruta 1962, Nasrallah and Wallace 1968, Richards and Thurling 1973). Thus, although incompatibility in crucifers is principally controlled by a major gene with multiple alleles, an actual population may be made up of many individuals with different manifestations of incompatibility brought about by the interplay of genetic and environmental factors.

Detection of self-incompatibility.   In a breeding program to exploit hybrid vigor in crucifers, the identification of self-incompatibility among selected plants and their subsequent progenies is of prime importance for the breeder. The traditional method of detecting self-incompatibility

is through seed set analysis. Since, however, it takes more than a month from pollination to harvest, that inadvertent contaminations in pollination and seed mixing at harvest are hard to eliminate completely, and that other environmental factors may set in to influence the self-incompatibility reaction, several workers developed possible alternatives to the seed-set method.

Thompson and Howard (1959) suggested a technique of observing darkened stigma surface two days after pollination. This method is not readily applicable to Chinese cabbage which lacks conspicuous differences in its stigma surface color. Sampson (1964, 1967) developed a technique of counting empty pollen grain on the radish stigma 24 hours after pollination, which was subsequently applied to Chinese cabbage (Lee and Yoon 1981). The ability of the fluorescent microscope (FM) to readily display the pollen tubes that have penetrated the style provides a direct and immediate measure of incompatibility (Fig. 11). FM is commonly employed in *Brassica* breeding (Wallace 1979), and has been combined with the *in vitro* pollination technique (Lee et al. 1982). Details of the FM method and its combination with *in vivo* pollination are as follows:

1. The newly opened flowers with pedicels are detached and neatly placed in a labeled petri dish with cover. Excised flowers are then pollinated *in vitro*. Inadvertent pollinations should be avoided. Pollinated flowers are kept in a chamber at RH 98% and 25°C for 24 hours. The humidity is maintained by sealing the chamber saturated with potassium dichromate $(K_2Cr_2O_7)$ solution within.

2. Pistils are removed and placed in 1 N NaOH at 60°C for about 45 min to soften the tissues, and then stained overnight with 0.2% aniline blue containing 2% potassium phosphate (K_3PO_4).

3. The stigma and style are mounted and then squashed on a microscope slide. The aniline blue stain accumulates in the pollen tubes and fluoresces when irradiated with ultra violet light at 300 to 400 micrometer wavelength. Under an FM microscope with appropriate light filters, the tubes are visible, whereas the background of stylar tissues is largely unseen. Penetration of the style by none or a few tubes indicates incompatibility; penetration by many tubes indicates compatibility; and penetration by intermediate numbers indicates intermediate strength of incompatibility expression (Fig. 11).
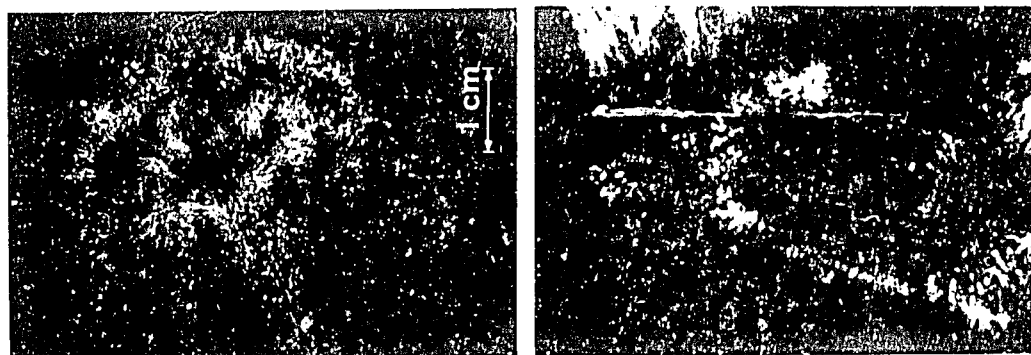
Figure 11. Fluorescence microscopic view of pollen tube elongation into the style of the compatible pollination (left), and of pollen grains with very short pollen tubes and strong fluorescence (callose formation) indicating incompatibility (right).

4. With *in vivo* pollination, pistils are excised 24 hours after pollination. Before softening and staining they can be stored by fixing in FAA (13 parts formalin, 5 parts glacial acetic acid, and 200 parts 50% ethanol), and then transferring to 70% ethanol.

When alternative systems fail, breeders generally fall back on the seed set method for detecting self-incompatibility (Fig. 12). The procedures used at AVRDC are as follows:

Figure 12.
Silique set in the raceme of a self-incompatible line. Open flower pollinations near the bottom result in small siliques with few or no seeds, indicating incompatibility; bud-pollinations at the top produce normal siliques, indicating juvenile compatibility.
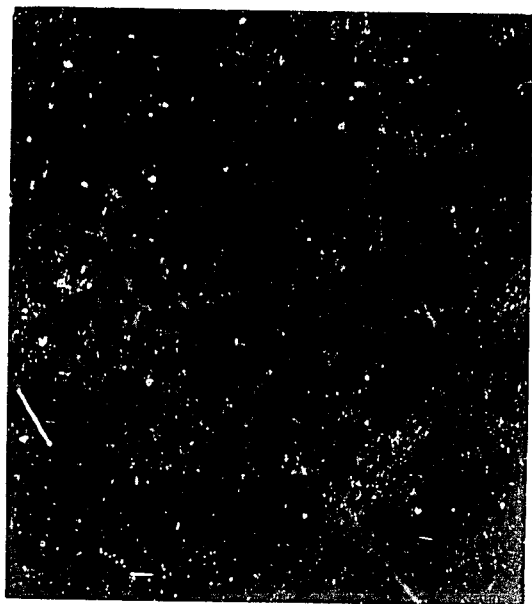
1. More than three flower stalks of the plant are selected and bagged to be tested for incompatibility. At an appropriate time, both open flowers, except very old flowers which are pinched off, and young buds, except the very immature ones, are self-pollinated. The number of self-pollinated open flowers and buds in each stalk are recorded in the pollination tag, along with the pedigree code of the plant and date of pollination.

2. At maturity, the number of seeds from open-flower selfing in each flower stalk are counted. The average seed set per silique (total seed set/total flowers pollinated) is then determined.

3. Self-pollinations averaging no more than one seed per silique in open-flower selfing are considered as good indications of self-incompatibility. This standard is, however, arbitrary and may differ depending upon the circumstances. For instance, in situations where some degree of sibbing in commercial seed lot is acceptable, the standard could be set a little higher. However, breeders generally follow the standard of one seed per silique or less on open-flower selfing to minimize sibbing problems during the production of commercial hybrid seed.

4. The average seed set on bud selfing is also determined to insure that the plants selected for strong incompatibility can also be easily maintained. In this regard a strict standard is not followed, but any appreciable deviation from normal fertile pollinations are noted and lines with aberrant bud fertility are eliminated.

Isolation of S-allele homozygotes.   Developing inbred lines that carry all possible desirable traits, the homozygosity for S-alleles with strong and stable self-incompatibility, and at the same time, with good bud self-fertility, are important prerequisites in hybrid breeding.

Most self-incompatible selections from a freely interbreeding population will likely carry their S-alleles in heterozygous form. The first selfed generation of these selections will, thus, segregate for S-alleles. If such alleles are symbolized by affixing letters after gene S, a situation might be visualized in which a selfed heterozygote, S_aS_b for example, is theoretically segregating into: $1 S_aS_a : 2 S_aS_b : 1 S_bS_b$. Breeders need to differentiate these genotypes and carry on further inbreeding only on S-allele homozygotes (S_aS_a, S_bS_b or both). Since all of these genotypes are self-incompatible, the identification procedure must involve testcrossing. A diallel-crossing method has been used at AVRDC; its theoretical bases and guidelines for practical application are described below.

Interaction types and diallel cross-fertility patterns: There are basically four *S*-allele interaction types in a sporophytic SI system (Fig. 13). Depending on the type, it is possible to identify homozygotes for *S*-alleles through a diallel-mating system. These homozygotes should be identified as early as possible in the inbreeding process on plants showing good combining ability and the lines should be perpetuated thereafter from the *S*-allele homozygotes.
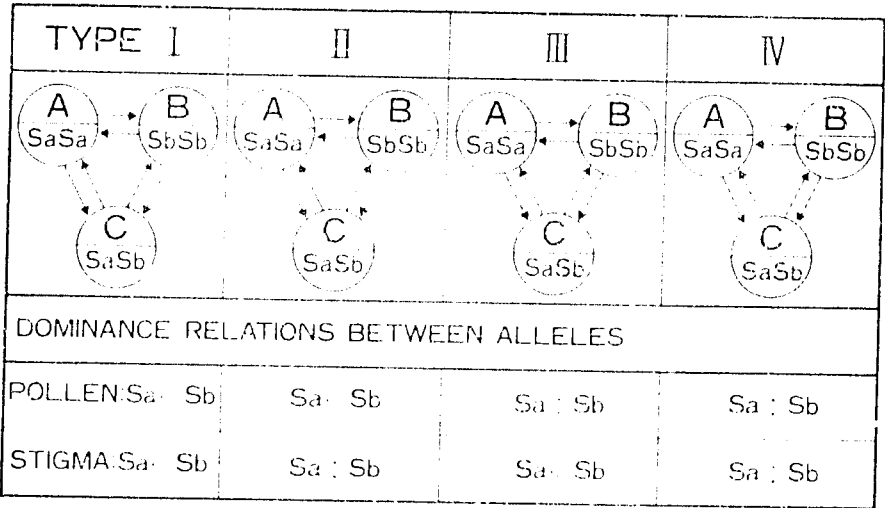
TYPE I	II	III	IV
DOMINANCE RELATIONS BETWEEN ALLELES			
POLLEN:Sa·Sb	Sa·Sb	Sa : Sb	Sa : Sb
STIGMA:Sa·Sb	Sa : Sb	Sa·Sb	Sa : Sb

Figure 13. Types of incompatibility relationships of S-alleles in pollen and stigma in crucifer vegetables. ---> cross incompatible, ---> cross compatible. Arrow indicates direction of pollination. $S_a < S_b = S_b$ dominant over S_a, $S_a:S_b = S_a$ and S_b independent.

The proportion of sibs (brother-sister mating) should be minimal or ideally zero in a hybrid seed bulk. This frequency will depend largely upon the strength of *S*-alleles and their stability in response to environment (especially temperature) for seed production. Selection for strong *S*-alleles can be facilitated after the identification of *S*-allele homozygotes.

The four basic *S*-allele interaction types and their cross-compatibility patterns are given below. Although it appears extremely simple on diagram, it requires careful and thorough analysis to arrive at the correct interpretation in practice. One must remember that the composition of the selfed progenies of a heterozygote only approximates the ideal 1:2:1 ratio. There could be more than one segregant of S_aS_a or S_bS_b, for example. Rearrangements of the diallel table of pollination data is, therefore, helpful to make the pattern clear. For explanation of the various symbols used, please refer to the notes below.

1. Type I: $S_a < S_b$ in stigma; $S_a < S_b$ in pollen

Genotype (phenotype) of seed parent	Genotype (phenotype) of pollen parent		
	S_aS_a (S_a)	S_aS_b (S_b)	S_bS_b (S_b)
S_aS_a (S_a)	I	C	C
S_aS_b (S_b)	C	I	I
S_bS_b (S_b)	C	I	I

Features:

a. There are more compatible crosses than in other types, about equal in number on each side of the diallel table.

b. The sibs can be divided into two sib-incompatible groups which are compatible to each other.

c. The group comprised of fewer plants likely consists of the recessive homozygotes (S_aS_a). The larger group is likely a mixture of heterogygotes (S_aS_b) and dominant homozygotes (S_bS_b).

d. S_bS_b is difficult to differentiate from S_aS_b (heterozygote) because of exactly similar cross-compatibility patterns. Appropriate test cross with a known heterozygote of previous generations, if they can be propagated vegetatively until the test to differentiate S_aS_b and S_bS_b genotypes, is suggested (Wallace 1979). In the case of Chinese cabbage, the test cross, however, is conducted in the next generation because of the difficulty in vegetatively maintaining parental lines.

2. Type II: S_a S_b in stigma; S_a < S_b in pollen

Genotype (phenotype) of seed parent	Genotype (phenotype) of pollen parent		
	S_aS_a (S_a)	S_aS_b (S_b)	S_bS_b (S_b)
S_aS_a (Sa)	I	C	C
S_aS_b (S_a and S_b)	I	I	I
S_bS_b (S_b)	C	I	I

Features:

a. Compatible crosses are more evenly distributed among columns than among rows.

b. The group of plants that are compatible as a female with the rest of the sibs but not reciprocally (as male) is homozygote S_aS_a.

c. The plants that are reciprocally compatible with S_aS_a are homozygous for the opposite allele, S_bS_b.

3. Type III: S_a < S_b in stigma; S_a S_b in pollen

Genotype (phenotype) of seed parent	Genotype (phenotype) of pollen parent		
	S_aS_a (S_a)	S_aS_b (S_a and S_b)	S_bS_b (S_b)
S_aS_a (S_a)	I	I	C
S_aS_b (S_b)	C	I	I
S_bS_b (S_b)	C	I	I

Features:

a. Compatible crosses are more evenly distributed among rows than among columns.

b. The group of plants that are compatible as male with the rest of the sibs but not reciprocally (as female) are homozygote S_aS_a.

c. The plants that are reciprocally compatible with S_aS_a are homozygous for the opposite allele, S_bS_b.

4. Type IV: $S_a = S_b$ in stigma; $S_a = S_b$ in pollen

Genotype (phenotype) of seed parent	Genotype (phenotype) of pollen parent		
	S_aS_a (S_a)	S_aS_b (S_a and S_b)	S_bS_b (S_b)
S_aS_a (S_a)	I	I	C
S_aS_b (S_a and S_b)	I	I	I
S_bS_b (S_b)	C	I	I

Features:

a. There are generally few compatible crosses on either side of the diallel table.
b. The plants that show reciprocal compatibility with each other are homozygous for each of the alleles, i.e S_aS_a and S_bS_b.

Notes:
a. The symbol in parenthesis of each diallel table, following or under each genotype, is the gamete and/or gametic behavior.
b. C = compatible; I = incompatible.
c. $S_a < S_b$ means S_b is the dominant allele; $S_a = S_b$ means both alleles are of equal strength and are therefore codominant.

   Additional guidelines in detecting S-allele homozygotes: In adapting the diallel-mating system, AVRDC breeders have followed certain 'rules of thumb' to insure the reliability and the effectiveness of the method in detecting S-allele homozygotes. These general guidelines are as follows:

1. In the diallel (with reciprocals) test of selfed progenies, it is recommended to use no less than 12 plants. This sample size provides about 95% probability that at least one of the S-allele homozygotes would be detectable.
2. For each cross or selfing, pollinate at least six open flowers; do not pollinate very old flowers, nor closed buds, as they tend to be more compatible and do not express the true self-incompatibility behavior. In practice the greater the number of pollinated flowers, the more reliable will the seed set data be. However, vigor of the individual plants often limits the number of flower stalks that can be used. Preferably pollinate only those flowers within the period from the day of, to the day after anthesis. To detect the seed set potential of each plant, a check cross using bulked pollen from an open-pollinated variety may be added.
3. With flourescent microscope it is possible to detect the self-incompatibility behavior quickly by observing pollen tube growth in the style. Without FM collect the data on the average number of seeds per silique (in this case the total number of seeds obtained from pollinating six flowers). While the limit of what may be considered incompatible pollination depends upon a particular situation, it is usually advantageous to select for strong SI to insure higher hybridity in hybrid seed production fields. To achieve this consider only those pollinations with an average of less than one seed/silique as incompatible. Anything above the limit should be considered provisionally compatible or fertile, depending upon how the average seed set compares with the check cross.
4. In the absence of a quick method for SI detection, each plant must be bud-pollinated for its own perpetuation. Data on the average seed set from bud pollination are also important in selecting good, easily maintainable lines. It is not sufficient for a line to exhibit strong self-incompatibility; the line must also be easy to maintain. This can only be achieved by selecting a line with strong SI and at the same time good bud self-fertility.

5. Homozygosity for an *S*-gene is unequivocally confirmed by the absence of fertile or compatible crosses among its selfed progenies when intercrossed in diallel (with reciprocal) fashion. After identifying the homozygotes, continue to monitor their selfed progenies by exploratory sibbing and selfing, i.e. incomplete diallel. Perpetuate the line only from the most self incompatible, highly bud self-fertile progenies. If the line is questionable for *S*-allele homozygosity, reexamine the line to isolate the true homozygotes. Otherwise, discard lines unstable for self-incompatibility even after unequivocal homozygotes have been identified. Most likely, there are modifiers influencing the expression of *S* alleles.

6. A line that shows complete reciprocal incompatibility, i.e. no compatible crosses in the diallel table, is homozygous for an unknown *S* allele and interaction type. Such a line is still useful where the immediate goal is a single-cross hybrid.

Male Sterility

The sporophytic SI system is the regular mechanism in crucifers to maintain heterozygosity and preserve their aeterogeneous population structure. From a plant improvement viewpoint, this mechanism has played a vital role in the exploitation of hybrid vigor in crucifers. Until now, hybrid varieties in a number of cruciferous vegetables continue to be produced using this system. Although this system is widely adopted, there are many concomitant problems involved in its use (see Surmounting the incompatibility Barrier and Insect factors in hybridization, p. 62 and p. 64, respectively). Eventually, these problems translate into high seed costs to growers. Plant breeders have, therefore, been greatly interested in finding alternative schemes for hybrid production — ideally, one that is more stable, simple and straightforward, and at the same time more economical than the sporophytic SI system. This search is set apart from efforts that have been expended and will continue to be spent in refining the use of self-incompatibility as the standard system for hybrid seed production (see Maintenance and multiplication of SI parents, p. 61).

The emphasis on exploitation of cytoplasmic male sterility as a possible alternative to self incompatibility has gained momentum in recent years. Genic male sterility, discovered earlier in certain taxa, has also received some attention and has actually been utilized for commercial hybrid seed production in some countries.

Genic male sterility. Genic male sterility is manifested through the nuclear genes inhibiting the normal development of anthers and pollens (Fig. 14). The precise stage at which pollen development is interrupted may differ with the species, or with the specific gene for male sterility within the species. The effectiveness of a male sterile gene may be measured by (1) the percentage of pollen grains that are viable, or (2) the percentage of seed set. The expression of a particular gene may be complete, so that there will not be any viable pollen or seed set in male-sterile flowers provided they have been protected to exclude pollen from external sources. Or, the expression of the gene may be partial permitting small amounts of viable pollen and seed set. The expression of the gene may also vary with the environment. Unless a male-sterile gene inhibits virtually all seed production, and is stable in a wide range of environments, its utility in breeding programs would be limited.

The first case of genic male sterility was reported in Japanese radish (*R. sativus*) by Tokumasu (1951). This genic male sterility is governed by a pair of recessive genes, *msms*; the dominant alleles (*MsMs* or *Msms*) result in production of normal anthers and pollen. Since then, several cases of genic male sterility have been found in other crucifers: broccoli (Anstey and Moore 1954); common cabbage (Kototani and Yamada 1958, Nishi and Hiraoka 1957, Rundfeldt 1960); Brussels sprout (Johnson 1958, Nieuwhof 1961); cauliflower (Borchers 1966, Nieuwhof 1961, Rundfeldt 1960); *B. campestris* var. brown sarson and yellow sarson (Chowdury and Das 1966, Das and Pandey 1961); *B. napus* (Heyn 1973, Takagi 1970); and Chinese cabbage (Anonymous 1978).
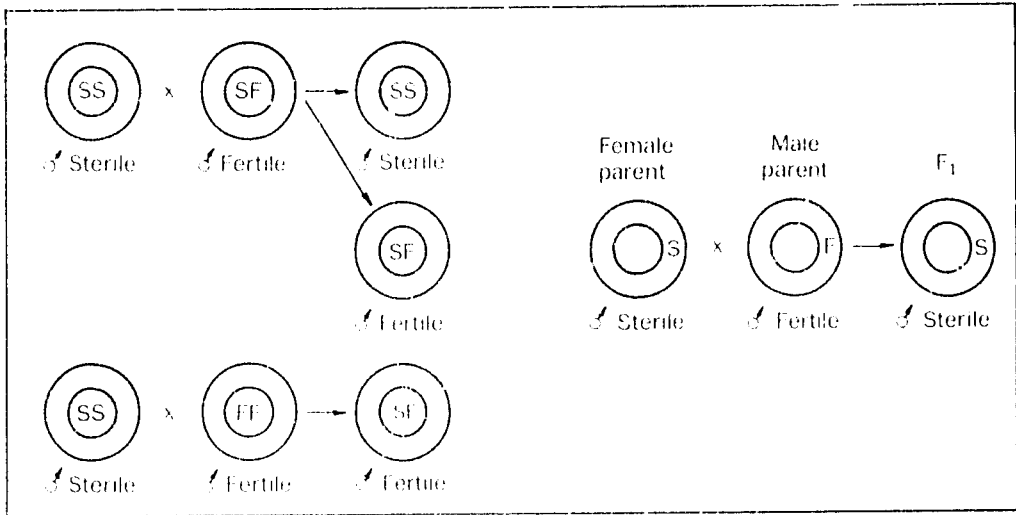
Figure 14. Mode of inheritance of genic male sterility (left) and cytoplasmic male sterility (right). Letters in the inner circles represent genetic factors; letters in the outer circles show cytoplasmic factors. S = male sterile, F = male fertile, and gene F being dominant over gene S. The cytoplasmic factors are transmitted only through the female parent (modified from Allard 1960).

F₁ hybrid Chinese cabbage has been produced in China using single recessive genic male steriles (Anonymous 1978, Niu et al. 1980). The system has many advantages: stock seed propagation is easily accomplished by sib-mating sterile plants with fertile plants of the same line; the quality of hybrid seeds is ensured; and their parents are more vigorous (Niu et al. 1980). Furthermore, male-sterile plants can usually develop normal fertile flowers at a low temperature (Nieuwhof 1968b). However, hybrid seed production, through genic male sterility, is an inefficient system because a pure population of genic male-sterile plants cannot be produced; hand-roguing of heterozygous male-fertile plants from female lines is required prior to the onset of bee pollination. Even assuming that an effective system for selecting out fertile plants from female lines can be found, e.g. seedling genetic marker closely linked to male-sterile gene, the system does not provide for a cost effective seed production since only half of the female population participate in producing hybrid seed. The relative interest, therefore, in the use of genic male sterility for hybrid production is limited.

Cytoplasmic male sterility. Cytoplasmic male sterility is controlled by the cytoplasm, but may be influenced by the nuclear genes (Fig. 14). Like genic male sterility, it results in the production of flowers with nonfunctional anthers or pollen (Fig. 15). The sterile cytoplasm often results from the introduction of nuclear chromosomes into a foreign cytoplasm. Since the cytoplasm is transferred only through the egg, cytoplasmic male sterility (CMS) is transmitted only through the mother plant.

Ogura (1968) reported the first case of cytoplasmic male sterility in Japanese radish. This male sterility was produced by the interaction between the cytoplasm and the homozygous nuclear gene, *msms*. Subsequently, other cases of CMS have been found: in *B. oleracea* from *B. nigra* × *B. oleracea* (Pearson 1972); in *B. campestris* subsp. *chinensis* from *Diplotaxis muralis* × *B. campestris* (Hinata and Konno 1976); in *B. campestris* subsp. *rapifera* (Ohkawa 1985); in *B. napus* (Shiga and Baba 1973, Thompson 1972); and in *B. juncea* (Brar et al. 1980). In some cases fertility-restoring factors have been found, while in others they were absent (Ohkawa and Shiga 1981, Shiga 1980). In the presence of a dominant fertility-restoring allele, the sterile cytoplasm becomes inoperative and the anther produces normal pollen while in the presence

of the contrasting recessive alleles, male sterility is expressed. To overcome the lack of an efficient genetic restoration of CMS, the use of ethrel, applied at 2000-3000 ppm before the emergence of the first flowering shoots in *B. juncea*, has been suggested (Banga and Labana 1984).

The possibility of hybrid seed production using CMS in *B. campestris* was extensively reviewed (Ohkawa and Shiga 1981, Ohkawa 1985). The utilization of a cytoplasmic male-sterile line of *B. napus* in successive backcrosses to *B. campestris* to develop a male sterile line in the latter has also been suggested (Ohkawa and Shiga 1981). As *B. campestris* carries few or no fertility-restoring genes for the *B. napus* cytoplasm, the repeated backcrossing can be greatly facilitated. In an even better case, Ohkawa and Shiga (1981) proposed the use of already available male-sterile lines in turnip (*B. campestris* subsp *rapifera*) which also carry *B. napus* cytoplasm. In a repeated backcross program to transfer the male sterile trait to other subspecies of *B. campestris* (e.g. subsp. *pekinensis*), it will no longer be necessary to stabilize the chromosome number of the hybrids, as one would if the source of the male sterile cytoplasm belonged to another cytoplasmic group. However, care should be taken in order to facilitate the transfer that the recipient of the male sterile cytoplasm carries no restorer gene. The CMS approach should simplify hybrid seed production and could, thus, render the transfer of this technology to developing tropical countries more feasible.

The development of cytoplasmic male-sterile lines among heat-tolerant Chinese cabbage is being explored as a supplement to the sporophytic SI system for hybrid seed production at AVRDC. Initially, the Ogura cytoplasm derived from radish was used following the details of nuclear substitution transfer of the *B. campestris* genome provided by Williams and Heyn (1981). This substitution used the *B. napus* substituted male sterile stocks developed by Bannerot et al. (1974) as intermediate parents. The result, however, has been discouraging due to problems of poor vigor, chlorosis, and other floral abnormalities, and partial to complete suppression of nectary development (Fig. 15) which inhibits normal bee activity and leads to minimal pollination and poor seed set. However, Leung et al. (1983) reported that the selection of lines with partially restored nectary development in cytoplasmic male-sterile *B. campestris* subsp. *pekinensis* and *chinensis* is possible. Since cytoplasmic male-sterile *B. campestris* is environmentally stable for sterility, CMS lines with resistance to diseases such as clubroot, turnip mosaic virus, and downy mildew were subsequently developed (Leung and Williams 1983). A few cytoplasmic male-sterile Chinese cabbage plants with functional inner nectar glands have been found recently at AVRDC. However, gland size is still smaller than those in the male-fertile recurrent parent. Moreover, their genetic stability has not yet been improved.

Another source of CMS, *B. juncea* cytoplasm, has also been explored by AVRDC breeders. A male-sterile *B. juncea* stock from India appears to be a better source than the radish-derived Ogura CMS because it exhibits normal nectary function, less chlorosis, and good plant vigor. This research which aims to determine whether *B. juncea* cytoplasm would be a more suitable alternative is still in progress.
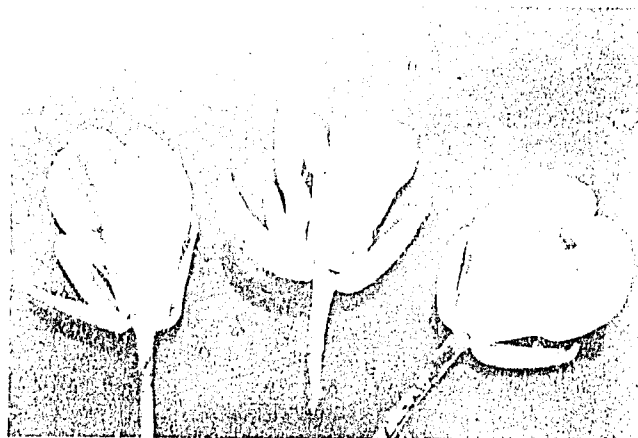

Figure 15.
Male sterile (both side) flowers in comparison with male fertile flower (center). Only degenerated vestigial stamens are seen in male sterile flowers, while they are normal in male fertile flower. Note also the suppressed nectar gland development in the male sterile flowers.

Breeding

Goals in Chinese Cabbage Improvement

Varietal improvement of heading Chinese cabbage has been most remarkable in Japan and Korea, where it ranks as one of the most important vegetables. Progressive refinements of breeding methods, especially utilization of self-incompatibility during the past 20 years, have substantially improved the yield of Chinese cabbage in Japan and Korea. In 1981 over 300 commercial cultivars developed through the efforts of public and private sectors, were on the market in Japan (Watanabe 1981). The number of registered Chinese cabbage cultivars in Korea has also increased considerably during the past 15 years (Pyo 1981). In China various types of Chinese cabbage have been released recently through the efforts of public research institutions (Jiang 1981). Much of the varietal improvement in Eastern Asia has been influenced by consumer preference, cultural techniques, cropping seasons, and pest incidence.

More than 15 years ago, AVRDC started its research program to improve the adaptation of heading Chinese cabbage in the tropics. As the hot, humid environment is beyond the normal ecological range for Chinese cabbage cultivation, new breeding objectives such as heat tolerance, early maturity, and disease resistance had to be pursued. These goals, as well as those commonly sought under the traditional temperate environment, are discussed below.

High yield, uniformity and earliness. These goals are grouped together because in an intensively grown crop like Chinese cabbage, they are premium characteristics that breeders generally look for. Much of the improvement in these objectives has been collectively achieved through the development of F₁ hybrids. The pronounced hybrid vigor obtained upon crossing two homozygous inbred lines makes possible the attainment of high productivity and in certain cases, also improved earliness. Similarly, the immediate progenies between two homozygous lines are virtually alike, genetically and phenotypically. Thus, the F₁ hybrid generally display excellent uniformity in appearance, maturity, performance, and quality. It is estimated that the yield of Chinese cabbage in Japan has doubled since 1960 as a result of breeding and distribution of excellent F₁ hybrid cultivars with disease tolerance (Matsumura 1981).

The AVRDC tropical Chinese cabbage program also pursues the above goals. High yield is always an important goal. Earliness and uniform maturity are also important traits that heat-tolerant cultivars grown during the hot, humid season must possess in order to reduce the risk of crop loss due to adverse weather conditions (Opeña and Lo 1981).

Resistance to major diseases. Chinese cabbage is attacked by several diseases, notably: softrot (*Erwinia carotovora*), downy mildew (*Peronospora parasitica*), mosaic virus, clubroot (*Plasmodiophora brassicae*), Alternaria leaf spot, and a few other minor diseases (Color plates B and C, p. 43 and p. 46, respectively). The importance of these diseases varies depending on the environment. In temperate countries, breeders have traditionally worked on resistance to softrot, downy mildew, turnip mosaic virus, and clubroot. With the exception of softrot, these diseases may be considered as cool-season diseases. In the tropics the first three are the most important. In some highland tropical areas, Alternaria leaf spot is a serious problem during the rainy months. Moreover, clubroot has become widespread in recent years in some of these locations, especially in the highlands. Genetic resistances to some of these diseases are available and breeders have, in certain instances, successfully incorporated them into commercial cultivars.

In recent years, soilborne diseases caused by *Aphanomyces raphani* and *Verticillium* spp. have become more serious in the major temperate production areas where successive Chinese cabbage cropping is common. Black rot caused by *Xanthomonas campestris* is also likely to become a serious problem under rainy, humid conditions as heading Chinese cabbage gains popularity in the tropics. Sclerotinia rot (*Sclerotinia sclerotiorum*), a perennial problem during seed production, is also becoming a common disease under cool, wet conditions in the commercial production areas.

Three different viruses are known to cause the mosaic disease of Chinese cabbage: turnip mosaic virus (TuMV), cucumber mosaic virus, and cauliflower mosaic virus. Efforts have been made at AVRDC, and other research institutions, to identify and incorporate resistance to TuMV the most widespread among the viruses, into Chinese cabbage.

Apart from diseases there are distinct physiological disorders, e.g. tipburn and internal rot, which are caused by unfavorable environments and/or unbalanced uptake and distribution of calcium and boron (Color plate D, p. 46).

Tolerance to environmental stresses.   Heading Chinese cabbage is generally less hardy than other *Brassica* crops like cabbage and its allied forms; it is more prone to environmental fluctuations. Under the high temperatures of the tropics, head formation of traditional temperate cultivars is either absent or loose. The development of heat tolerance in this crop has, therefore, been a major goal at AVRDC.

Under protracted cold temperatures, Chinese cabbage tends to go to flowering easily. In temperate countries, bolting resistance is an important trait to enable prolonged Chinese cabbage supply towards the winter period or early availability in the spring season. Bolting resistance is also an important trait in the tropical highlands where temperatures may be low enough to cause bolting, especially among heat tolerant varieties.

Quality and preference traits.   Although considerable genetic variation is known for some quality traits such as vitamin C content and thiocyanate content (Daxenbilicher et al. 1979, Park and Kim 1985), the quality aspects of Chinese cabbage rarely have become a major concern in the tropics. Dark green cultivars generally contain higher levels of vitamins than light green ones. This trend is true between plant parts and different genotypes. Lower thiocyanate content is an important character due to its association with low pungency which is a desired trait when Chinese cabbage is grown under high temperature and low humidity (Park 1981).

Certain preference traits may play a key role in popularizing commercial varieties. In Taiwan, the round-shaped, smooth-leaved, wrapped over type of heading variety with dark green, smooth outer leaves are highly preferred by consumers during the summer. Sessile leaves with wide and flat midrib is also a premium character. However, totally different preferences are found in different seasons in the country.

Preference also differs between countries or regions, partly depending upon the types of cultivars that have been popularized in the past. In many Southeast Asian countries, cylindrical head shape is preferred to round shape.

Preferred head size also differs from place to place. Specific preferences appear to be related with the traditional methods of cooking or preparation, common shipping or marketing methods, etc.

From the breeders' viewpoint, it is important to assess the market preferences and even to foresee the changing trends in order to make the new cultivars more popular and acceptable.

Advances in Tropical Chinese Cabbage Research

Genetical and breeding aspects of heat tolerance.   The optimum mean temperature range for head formation of heading Chinese cabbage is $15°$ to $20°C$. In the lower latitudes of tropical Asia, this vegetable is usually produced in the cool highlands (Opeña and Lo 1979).

The criterion for heat tolerance in Chinese cabbage has been defined in terms of compact head formation under high temperatures (Opeña and Lo 1979). Screening for this special trait at AVRDC involves field evaluation during the hot, wet season, from May to September, during which minimum temperatures are normally above 21°C. Evaluation of the AVRDC Chinese cabbage germplasm for heat tolerance (Fig. 16) and other major traits required for the hot, humid areas has enabled the selection of elite genetic stocks for breeding (Opeña and Lo 1979, Opeña 1985).

Figure 16.
Cross section of heat-tolerant (left) and heat-sensitive (right) Chinese cabbage plants grown under high-temperature conditions. Head formation fails in the heat-sensitive one.

Heat tolerance in heading Chinese cabbage is inherited in a relatively simple fashion (Opeña and Lo 1979, Opeña and Lo 1981, Opeña 1985, Yoon et al. 1982), thereby rendering a straightforward genetic transfer. However, other important characters like head yield and disease resistance need to be combined with it in the breeding program.

The breeding philosophy to derive high-yielding, heat-tolerant varieties of Chinese cabbage has been extensively discussed (Opeña and Lo 1981, Yoon 1987). Fundamentally, the key to achieving high yield depends greatly on broadening the genetic base for heat tolerance to enhance heterosis. The genetic diversity among the tropical, heat-tolerant gene pool was found to be narrow and to 'enrich' such genetic base they were, therefore, crossed with unrelated cultivars carrying other important traits, such as disease resistance. Populations developed through mass selection from these intercultivar crosses served as sources of new heat-tolerant inbred lines for development of F₁ hybrids and/or open-pollinated populations. Hybrid vigor for head weight, the principal component of yield, among crosses of these new inbreds was substantial (Opeña and Lo 1981). High-yielding open-pollinated populations were also synthesized from intercrosses among the new inbred lines.

Yield improvement in the new tropical Chinese cabbage cultivars developed at AVRDC has been appreciable. Whereas old local cultivars, previously grown by farmers during the summer season in Taiwan, normally yield about 8-10 t/ha, 32-35 days after transplanting, the new hybrids and open-pollinated varieties can yield three times as much in the same growth period (Opeña and Lo 1979).

Some hybrids and open-pollinated varieties from AVRDC have been officially released in Taiwan, the Philippines, Japan, and China. In countries like Korea, the national programs have utilized AVRDC's inbred lines in developing locally adapted hybrids. The performance of the improved heat-tolerant Chinese cabbage has also been outstanding in other tropical countries, although the problem of seed supply has been a persistent dilemma preventing their outright release.

Physiological bases of heat tolerance.   The heading process in Chinese cabbage plays a major role in deciding yield under high temperatures (Kuo and Tsay 1981, Kuo et al. 1988).

Leaf turgidity is a prerequisite for leaf erection, hooking, and eventual head formation (Kato 1981, Kuo and Tsay 1981). Heat-tolerant cultivars maintain leaf turgidity under high temperatures (Kuo and Tsay 1981); head formation at high temperature relies more on the plant's water balance rather than on its photosynthetic source (Kuo et al. 1988). Heat-tolerant varieties utilize more water than heat-sensitive ones at leaf erection and hooking stages. This difference is due to the rapid root growth and extensive root system of heat-tolerant genotypes. In fact, all heat-tolerant varieties had lower shoot root ratios than heat-sensitive varieties (Kuo and Tsay 1981). The extensive root growth in heat-tolerant varieties may facilitate greater water uptake. Their initially high water uptake may conceivably be required to enable high turgor in the leaves so that heading can proceed normally at high temperature.

Furthermore, available data support the importance of thick leaves for head formation of heat-tolerant varieties (Kuo and Tsay 1981, Kuo et al. 1988). The absence of adequate drought avoidance in the form of a surface barrier of low water permeability, due to high stomatal number in the leaves of heat-sensitive cultivars, may contribute to their rapid dehydration and inability to maintain turgor at high temperature. Less leaf surface area per unit shoot weight of the thick-leaved varieties may also decrease transpiration and consequently improve the water economy of the plant.

Heat-tolerant Chinese cabbage plants contain more electrolytes than heat-sensitive ones (Kuo et al. 1988). A lower osmotic potential is also responsible for maintaining turgor when the water loss occurs due to high temperature. Thus, high electrical conductivity, due to high concentration of electrolytes in the leaf sap of heat-tolerant Chinese cabbage plants, should lead to the maintenance of higher turgor with a plentiful water supply. If turgidity is well maintained, initial heading processes, such as leaf erection, should proceed normally. High chlorophyll content in the outer leaves of heat-tolerant Chinese cabbage plants may also facilitate the photosynthetic rate, thereby increasing carbohydrates for use as the source of electrolytes or energy for the heading process.

The most important protective mechanisms that Chinese cabbage plant have adapted for head formation under high temperatures are summarized in Fig. 17. Some of these protective mechanisms (e.g. increased leaf resistance which reduces photosynthesis; decreased osmotic potential which reduces availability of photosynthate; or increased ion uptake which reduces energy availability) are ironically also detrimental to high productivity; thus, the average head size appears smaller under high temperature than under low temperature conditions.

It is reasonable to surmise that heat tolerance in Chinese cabbage is strongly associated with the water relations of the plant. Conventional psychrometry to measure the water status and water potential is not suitable for Chinese cabbage, however, so an indirect method like character association might offer a viable alternative to select for heat tolerance. The relevant morphological traits to aim for in selecting for heat tolerance are: (1) thick and dark leaves, and (2) vigorous root growth. These are essential in sustaining leaf erection for head formation under high temperature. Other traits showing association with heat tolerance may not be generally inferred as such because the heat-tolerant materials in these studies were almost exclusively from Taiwan which had been previously selected for the locally preferred morphology.

Resistance to major tropical diseases.   Varietal differences in reaction to artificial softrot infection have been observed; however, no reliable source of resistance has been successfully exploited thus far at AVRDC. An acceptable alternative to softrot resistance was achieved by developing early-maturing cultivars which are able to escape the disease. Many AVRDC hybrids, such as hybrids 58, 62, 82-156, and 82-157 are early maturing and heat tolerant, enabling them to escape softrot infection and to perform well during the hot, humid season.

The development of TuMV-resistant genotypes had depended previously on complex infection until a specific strain concept was established. Five distinct strains of TuMV have been recognized so far, and resistance to TuMV strains C-1, C-2, and C-3 among Chinese cabbage germplasm has been identified (AVRDC 1981). On the other hand, resistance to strains C-4 and C-5 in Chinese cabbage is rare, having been observed only in B 730 (AVRDC 1981, AVRDC 1985).
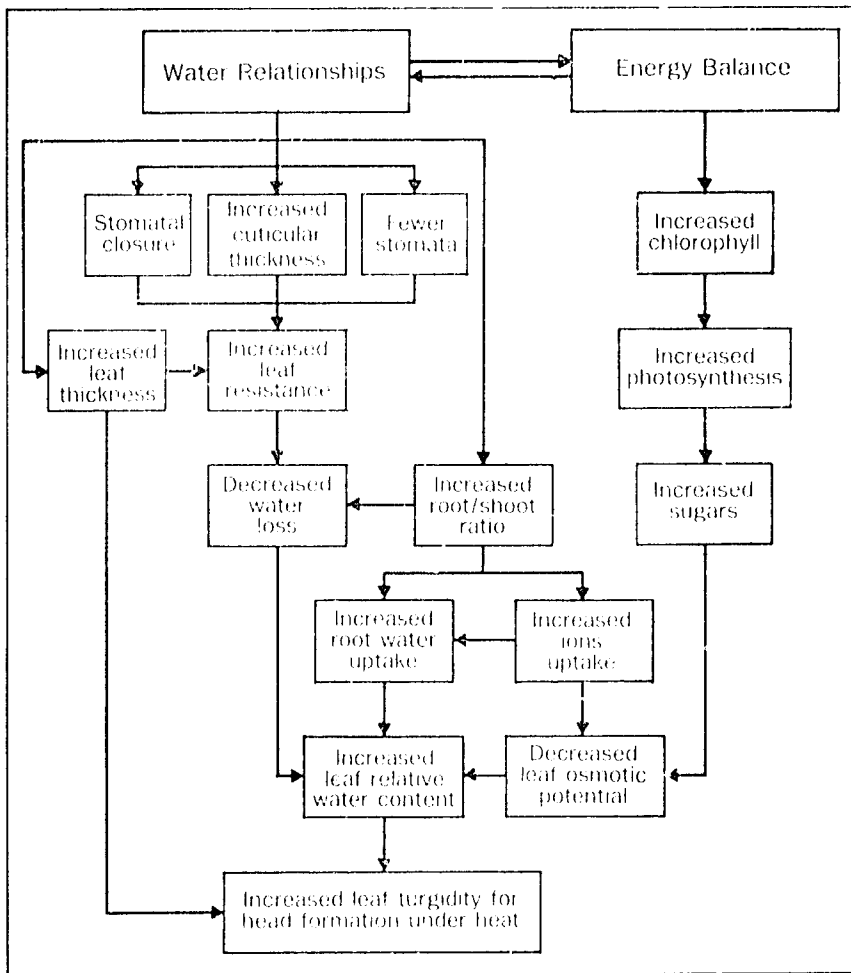
Figure 17. Protective mechanisms against heat stress during heading stage of Chinese cabbage.

Another useful accession, B 708 (PI 418957), carries immunity to strains C-1 and C-3 and resistance to C-2 and C-4 (AVRDC 1985).

A considerable level of resistance is achieved by regularly exposing accessions, breeding lines and segregating populations to downy mildew (DM) epiphytotics, either under field conditions, *in vitro* inoculation, or both. An *in vitro* technique using sporangia production on detached cotyledons from the seedling stage, inoculated and incubated for 12 hours at 14°C, promises to be a useful tool to screen a large number of seedlings under controlled environment (AVRDC 1985). The most promising sources of DM resistance used at AVRDC were Korean introductions, particularly B 742 (AVRDC 1981), B 639 (Hakuran), a synthetic amphidiploid between *B. campestris* subsp. *pekinensis* and *B. oleracea*, has also shown excellent DM resistance, and has been the important donor parent in a backcross program to transfer the resistance to *B. campestris* subsp. *pekinensis* (AVRDC 1985).

Some of AVRDC's hybrids, e.g. 58, 59, and 62, as well as open-pollinated populations like 77 M(3)-27 and 77 M(3)-35, carry varying levels of resistance to downy mildew, whose inheritance is polygenic, and turnip mosaic virus (AVRDC 1981). In some cases, the hybrids offer better and more durable field resistance levels, particularly to downy mildew, than local cultivars (AVRDC 1986, AVRDC 1987b).

Pollination Techniques

In Chinese cabbage breeding, it is necessary to artificially control pollination in order to achieve the desired type of mating. The forms of pollination control include emasculation and bagging of pollinated stalks, spatial isolation, or isolation using greenhouse, nethouse or movable net cages. In all forms the main purpose is to avoid pollen contamination so that only the desired type of mating takes place.

Preparations for pollination.   The plant material and tools needed for pollination, and the right temperature for pollen storage are discussed below.

Plant material: It is very important to secure an appropriate number of inflorescences of reasonable vigor to complete pollinations of different combinations on the same plant. Pinching of main stem and primary branches allows the primary and secondary branches to grow vigorously. The optimal time for pinching is at the onset of flowering of the flowering stalks to be removed.

In any given genotype, the earlier the plant is subjected to vernalization, the less vigorous the plant becomes. The vernalization treatment, therefore, has to be scheduled carefully to obtain vigorous female plants that can support normal flowering and seed development of all their pollinated branches. Pollen sources have to be secured at or before the flowering peak of the female plants.

Pollen storage: *Brassica* pollen, which has a thick pigmented pollen coat surrounding the grain, usually have a water content of 18-22%. It remains viable for about one week at 20 C if stored in a small closed glass container (Dumas et al. 1983). Pollen quality generally diminishes rapidly at room temperature. Numerous attempts have been made to store pollen under conditions that will maintain its viability for breeding purposes. However, the factors influencing the viability of *Brassica* pollen have proved elusive to understand and control satisfactorily. Furthermore, there is a great difference between pollen viability and the ability of pollen grain to fertilize; the capacity of a pollen grain to fertilize is probably better immediately after its dispersion rather than several days after storage.

At AVRDC Chinese cabbage pollen is collected using honeybee thoraxes which have been previously cleansed with tap water and sterilized by 70% ethanol following procedures described by Williams (1981). The pollen-bearing thoraxes are then put in a sealed jar containing silica gel as a desiccating agent. This jar is then stored in a freezer at -10 to -12 C. Through this means, pollen can be stored without drastic degeneration in viability for three to four months. Before using the pollen for pollination, the container is moved out of the freezer and kept in a refrigerator at about 5 C for several hours and then at room temperature for 30 min or so.

Tools: Label, pollination bag, forceps, pencil, methyl alcohol, and cotton wad (in petri dish) are essential supplies required at pollination time (Fig. 18).

Selfing.   Selfing is essential to develop inbred lines that are uniform for many horticultural traits, and homozygous for S-alleles. This mating scheme also provides full-sib progenies for testing in association with some selection methods to improve open-pollinated cultivars.

The flowering stalks to be selfed are bagged after removing the old flowers that may already have been contaminated with foreign pollen (especially if flowering plants are not kept in isolation). Self-pollination may be undertaken immediately or a few days thereafter, depending upon the purpose of selfing.

Without consideration of self-incompatibility: If selfing is done solely to produce selfed seeds regardless of tests for other factors such as self-incompatibility, one may immediately open the young buds to expose the stigma for outright pollination. A pair of sharp-pointed forceps
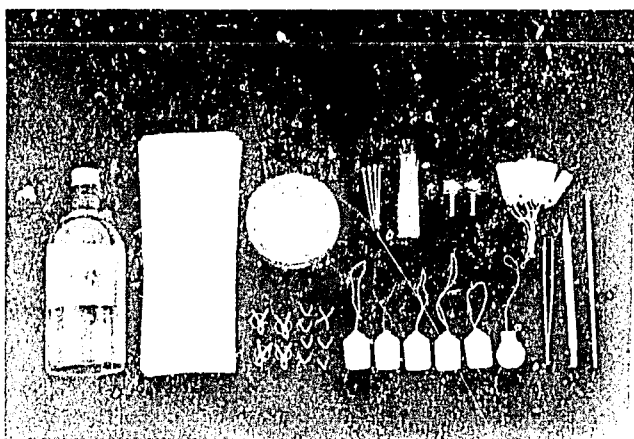
Figure 18.
Tools commonly used in emasculating and pollinating Chinese cabbages. From top left to bottom right are: (1) alcohol bottle; (2) paraffine bags; (3) petri dish containing cotton ball impregnated with alcohol; (4) clips; (5) toothpick with bee thorax; (6) glass ball; (7) labels of different color and shape; (8) forceps; (9) pencil and (10) brush.

facilitates the opening of buds. If a 'clean' source of pollen of the same plant is already available (either from storage or fresh from the plant), the newly opened buds may be pollinated right away by using a soft brush dipped in the pollen mass or brushed against fresh flowers with dehisced anthers. Alternatively, one may collect a dehisced anther with forceps and rub it lightly against the stigma. The pollinated stalks are then covered with a waxed paper bag (or glassine bag), secured by a clip and properly labeled as to its identity, date of pollination, type of pollination etc. The bags may be removed once the siliques have sufficiently developed, after which no obvious fertilization of the exposed stigmas can occur.

Very small buds have poor seed set potential and should not be forced open. To avoid confusion, the tip of the flower stalk bearing the immature buds that need not be pollinated should be pinched off with forceps.

With consideration of self-incompatibility: If selfing is intended to produce selfed progenies and at the same time to examine the self-incompatibility behavior of a plant, the bagged flower stalks should be allowed to develop for several days so that more fresh, open flowers are available. Self-incompatibility is strongest on flowers that are from a day old to a day away from opening but this age range can be influenced by temperature and humidity (see Modifications of self-incompatibility expression, p. 23). The general procedures of selfing are similar as above except that open flowers are included in the pollination. Freshly opened flowers are generally used also as a pollen source. Very old flowers and exceedingly young buds are pinched off from the inflorescence. Again, bag the selfed stalks. In the pollination tag, additional information on number of selfed open flowers and number of selfed young buds is included. The seed yield data on open flower pollination and bud pollination indicate the plant's level of self-incompatibility and relative bud fertility, respectively (see Detection of self-incompatibility, and Isolations of S-allele homozygotes, p. 26 and p. 28, respectively).

Crossing.   Flower stalks in the two parents designated for hybridization are bagged to avoid contamination by foreign pollen. This is especially advisable if flowering plants are not kept in isolation rooms. Again, open flowers of both parents are pinched off prior to bagging. In the intended female, unopened buds are emasculated by pulling off the six anthers with the aid of forceps. Immature buds are also pinched off. Likewise, buds that are about to open are avoided. If 'clean' pollen grains from the intended male are already available, pollination may be done soon after emasculation. Otherwise, the emasculated flower stalks may be bagged and pollinated the next day when pollen grains from the male parent become available; alternatively, one may forego with emasculation and pollination until appropriate flowers are available from both parents. Again, the label should reflect the identity of the cross, date of pollination, etc. Bagging after pollination is necessary, even in isolation rooms, to avoid accidental contamination.
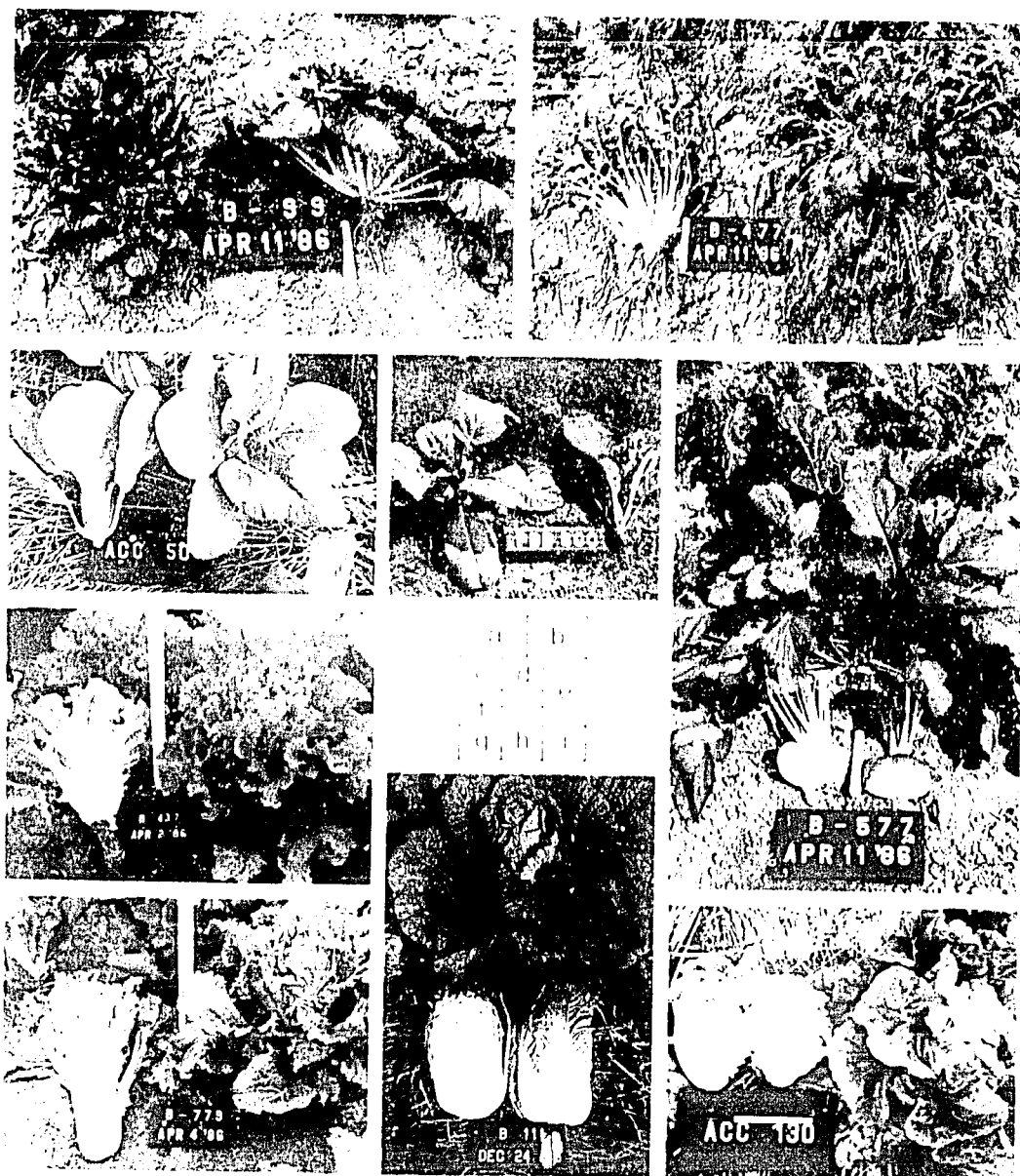
Color plate A Major subspecies of *Brassica campestris* (a) subsp *narinosa*; (b) subsp *japonica*, (c) subsp *chinensis*, (d) subsp *parachinensis*, (e) subsp *rapa*, and subsp *pekinensis* of (f) semi-heading, (g) cylindrical, (h) typical temperate, (i) typical tropical heading types.

Color plate B: Important fungal and bacterial diseases of Chinese cabbage (a) bacterial softrot;
(b) clubroot; (c) downy mildew; (d) Alternaria leaf spot; and Sclerotinia rot on
(e) head and (f) flowering stem

Although female parents used in hybridization may carry strong self-incompatibility, it is still advisable to emasculate them unless the purpose of crossing is to produce a small amount of F_1-hybrid progenies for combining ability tests.

Isolation methods.   Chinese cabbage is insect-pollinated and, therefore, seed production plots of different varieties must be spaced at least 1,000 m apart. This spatial isolation requirement is a major hindrance when many different stocks are being multiplied such as in a breeding program.

To avoid contamination plants for routine selfing or crossing may be kept in an insect-proof glasshouse or nethouse. Bagging of pollinated stalks can further reduce the chance of contamination. Movable insect-proof cages can also be constructed of nylon nets and these can be used repeatedly for small-scale selfing, crossing, or even production of hybrid seed in small lots. For relatively large-scale F_1 hybrid seed production involving several combinations, a semipermanent or permanent nethouse with suitable partitions is useful. The nethouse may also be used for other types of pollination. A partitioned glasshouse serves a similar purpose. These subdivided structures can also be used for large-scale maintenance of several inbred lines using bud pollination. If this is aided by gas treatment such as CO_2, a partitioned glasshouse is most appropriate.

In commercial hybrid seed production, spatial open field isolation is used to produce large seed amounts at low cost. Often, such seed multiplication is done on a community basis where seeds produced within a locality are of the same cultivar. Other techniques of isolation may be applied depending upon the circumstances.

Breeding Methods

Genetic improvement in crucifers takes much the same form as in other outcrossing species by virtue of their similarities in population structure and organization of genetic variability. The principal methods by which new varieties of cross-pollinated crops originate may be classified into four broad sources: (1) introduction; (2) selection; (3) hybridization; and (4) development of synthetic varieties.

Base materials for improvement may be open-pollinated cultivars coming from introductions, populations derived through hybridization, synthetic varieties, or other genetically broad-based stocks. The most fundamental requirement is that these breeding materials are genetically variable and possess the important characters that breeders aim to fix in the improved cultivars. Once these preconditions are met, the rest depends entirely on the breeders. This is where the breeder's skill in visualizing the ideal type (given the presence of an effective selection regime) and in applying the most appropriate selection methods usually come into the fore.

The selection methods commonly applied to crucifers are, as in other cross-pollinated crops, always based on a population concept. In cross-pollinated crops, individual plants are seldom used to establish a variety because segregation and cross-pollination make it impossible to maintain a distinct type; moreover, a wider range of genetic diversity than found in a single plant is generally needed to maintain a vigorous population. With the possible exception of early cauliflower varieties, crucifers generally suffer from inbreeding depression; therefore, techniques such as pure line breeding to develop cultivars for outright release is never undertaken except as a means of obtaining reproducible hybrids. The essential features of each of the selection methods are described below.

Mass selection.   The main purpose of mass selection is to obtain a high frequency of superior genotypes within the population. It is a selection procedure in which individual plants with desirable traits are chosen from the base population, and allowed to flower and interbreed together without control of pollination (Fig. 19). Seeds from these plants are then harvested in bulk without the benefit of a progeny test to constitute a new base population for selection. Mass selection
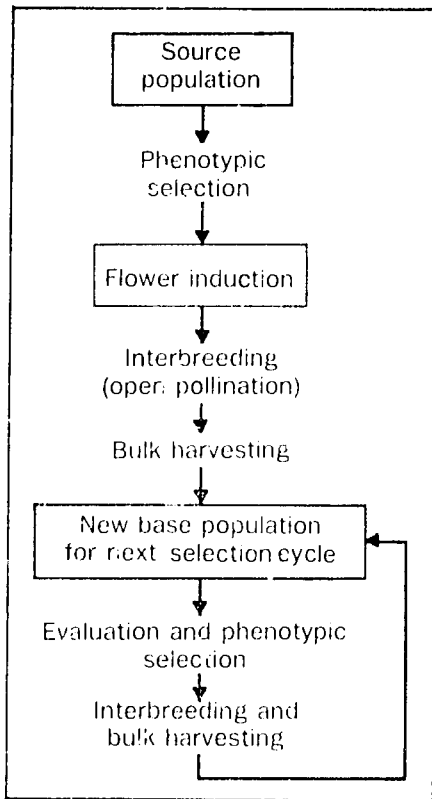
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┌─────────────────────────────────┐
│        ┌─────────────────┐       │
│        │     Source      │       │
│        │   population    │       │
│        └─────────────────┘       │
│                 ↓                │
│           Phenotypic             │
│            selection             │
│                 ↓                │
│        ┌─────────────────┐       │
│        │ Flower induction│       │
│        └─────────────────┘       │
│                 ↓                │
│           Interbreeding          │
│          (open pollination)      │
│                 ↓                │
│           Bulk harvesting        │
│                 ↓                │
│   ┌─────────────────────────┐    │
│   │   New base population   │◄───┐│
│   │ for next selection cycle│    ││
│   └─────────────────────────┘    ││
│                 ↓                ││
│     Evaluation and phenotypic    ││
│            selection             ││
│                 ↓                ││
│        Interbreeding and         ││
│         bulk harvesting ─────────┘│
└─────────────────────────────────┘
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Figure 19.
Schematic diagram for procedures of mass selection.

may be carried out repeatedly ('cyclical' mass selection) until the breeder deems it no longer worthwhile to carry on further selection, either because selectable variability has already been exhausted or, the achievable response to additional selection is no longer commensurate to the efforts. The seeds of a number of superior plants are then composited, planted in an isolated plot to mate at random, and then harvested in bulk to constitute the foundation seed of a new variety or new base population for further mass selection.

Since the merits of plants selected to contribute to the next generation is based solely on phenotypic appearance, mass selection is mostly effective in improving populations for highly heritable characters. If mass selection is to be effective, it is necessary that genetic variability exists within the mixed population. This technique is ineffective, however, to change populations for traits that are controlled by many genes, in which each exercises a small effect on the phenotype, and which are highly influenced by environment. Nowadays, mass selection is used largely to maintain cultivars.

The ineffectiveness of mass selection to improve traits with low heritability results from three main causes (Allard 1960): (1) inability to identify superior genotypes from the phenotypic appearance of single plants; (2) lack of pollination control so that selected plants are pollinated by both superior and inferior pollen; and (3) strict selection leading to reduced population size is tantamount to inbreeding depression. The second limitation above is not critical in crucifers because selection for the superior phenotypes may be done prior to flowering, and, therefore, intermating may be restricted among only the selected plants.

Mass selection is the simplest and perhaps, one of the oldest of the selection techniques. It is relatively simple for the breeders to select and composite seed from what appear to be phenotypically superior plants. Also, new varieties can be developed rather quickly. Since the improved strain will not differ greatly from the parent variety in the range of adaptation, less time is required for testing than with new breeding materials. Many varieties of cabbages and allied crops have resulted from mass selection.
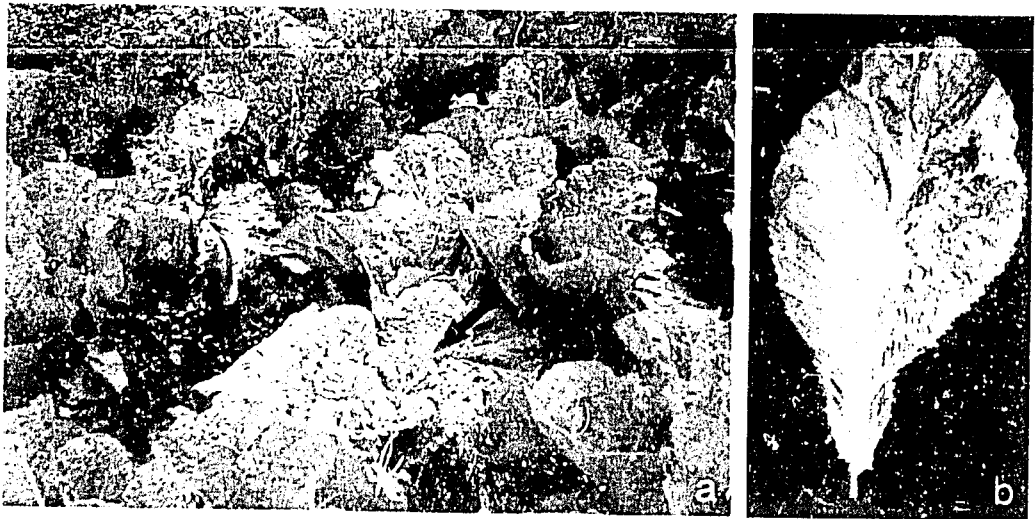
Color plate C   A virus-diseased plant (indicated by arrow) in the field (a); and mosaic (b) and ring spots (c) caused by turnip mosaic virus
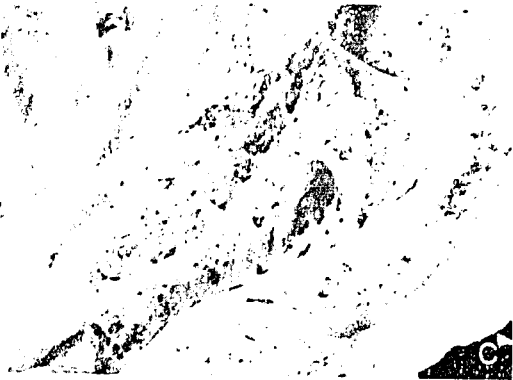
Color Plate D: Some physiological disorders of Chinese cabbage: (a) boron deficiency, (b) tip burn (marginal rot); and (c) internal rot

Family selection. As in mass selection, the selected plants are allowed to flower and interbreed together without control of pollination. In family selection, however, the open-pollinated seeds from each selection are harvested separately to constitute a 'family'. Thereafter, the families are planted side by side for comparison of their performance and only the best families are used in further selection. Plants selected from the best families are propagated together, especially if they resemble one another. A model for the family selection procedure is given in Fig. 20.
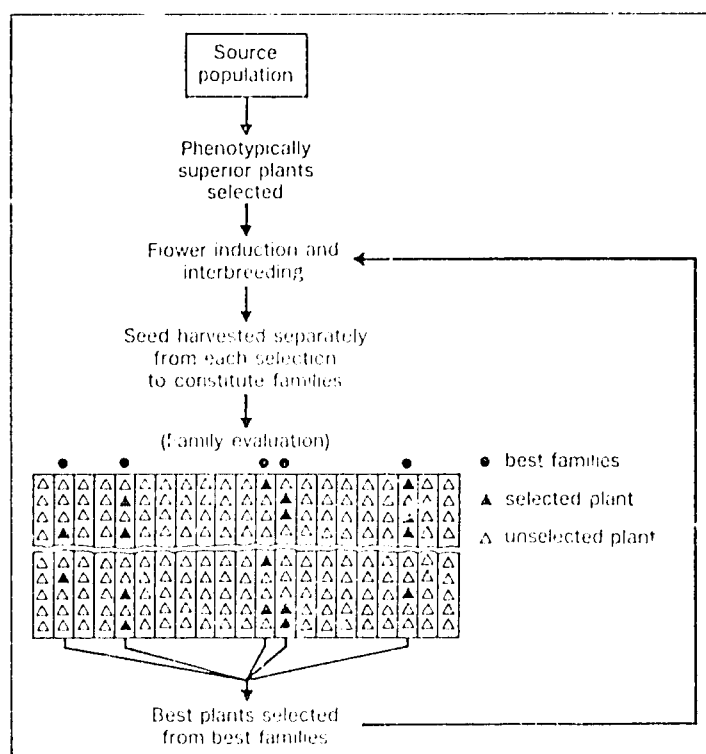
Figure 20.
Schematic diagram for procedures of family selection.

Since further selection is continued only among the progenies of genetically superior plants, family selection is more efficient than mass selection. If care is taken that sufficient numbers enter into every new generation, and that testing of progenies sufficiently negates the influence of environmental factors, family selection could adequately address most of the limitations of mass selection.

Family selection is as simple as mass selection and requires little special equipment. Applied to cole crops, it can give very good results. It can also be used to maintain varieties. For the commercial seed production of an open-pollinated variety, it is an attractive method.

Maternal line selection. Phenotypically superior plants are selected from the original population and allowed to breed *inter se* as in mass selection. At this stage, the best of the superior plants are planted in the center of the seed production lot. Seeds harvested from each of these best plants constitute a maternal line in the next selection cycle. Maternal lines are evaluated for their performance and individual plants are selected only from the superior lines; the best plants from the superior maternal lines are again placed in the middle of the seed-production plot where natural open pollination is allowed among the selected individuals. This method is, therefore, like mass selection in the pollen-parent selection phase, while somewhat similar to

family selection in the maternal-parent selection. A model for maternal-line selection procedure is illustrated in Fig. 21.

Maternal-line selection is effective for highly heritable traits and recommended in improving heterogeneous open-pollinated cultivars; considerable improvements in uniformity and early maturity of open-pollinated Chinese cabbages have been made with five to six cycles of this procedure (Shinohara and Sugano 1958).

Recurrent selection. In family selection, the superiority of selected individuals is judged from the performance of their open-pollinated progenies. It is generally agreed that a progeny test such as this provides a more accurate measure of the breeding value of an individual, rather than the phenotype of an individual itself.

The progenies for testing may be produced through different mating designs. They can be open-pollinated seeds of selected plants (as in family selection), selfed seeds of selected plants, or established through other forms of test crosses, e.g. topcross, polycross, diallel cross, paired cross, etc.

Many of the above techniques are very laborious and generally applied to crucifers only in limited circumstances; therefore, they are not discussed in this bulletin. However, the use of recurrent selection with topcross as a progeny-testing method is described here.

Recurrent selection, as its name implies, involves repeated selection on intervening populations derived from interbreeding of selected individuals in order to accumulate the desirable genes for a particular quantitative character without marked loss of genetic variability. The general procedure is to select from a genetically variable population the individuals that are superior for the character under consideration. The selected plants are selfed and test-crossed simultaneously (Fig. 22).

Selections to be interbred in order to constitute the next cycle are decided on the basis of the testcross performance. If a genetically broad-based tester (e.g. open-pollinated varieties) is used, recurrent selection is for the general performance of a strain in a series of crosses or 'general combining ability'. With a narrow-based tester (e.g. homozygous inbred line), recurrent selection is for the performance of a strain in a specific cross or 'specific combining ability'. On the other hand, reciprocal recurrent selection employs two broad-based populations as complementary testers and provides for selection of both general and specific combining ability.

The recurrent selection method adopted at AVRDC to improve the tropical Chinese cabbage open-pollinated cultivars involved phenotypic selection for large head size during the hot, humid season. Approximately 10% of each original population was selected. All selections were brought to flower in the greenhouse during the cool season, selfed and at the same time, each was crossed to an open-pollinated tester. The progenies from these test crosses were then evaluated for general performance (especially head weight and yield), in replicated plots. Based on topcross performance, only the best 20% of the initial selections were chosen to contribute to the next generation, giving an overall selection intensity of 2%. To interbreed the superior selections whose merits were judged from the general combining ability test, an equal number of selfed seeds per selection were mixed thoroughly, planted, brought to flower together, and allowed to interbreed. Bulked seeds from this isolated plot were then harvested *en masse* to constitute the next cycle seeds. The procedures were repeated for the next recurrent selection cycle.

Recurrent selection for general combining ability was effective in increasing the head weight of tropical Chinese cabbage although response was dependent on variety (Opeña and Lo 1981, Yoon and Opeña 1977). Some local varieties responded significantly to selection while others did not. Tropical Chinese cabbage cultivars, especially those collected in Taiwan, have undergone some form of selection by the farmers themselves, often in a rather reduced population size. Therefore, the variability necessary for further selection may have already been reduced, if not entirely exhausted, in some cultivars.

Backcross method. In some instances a well-known variety may lack a certain character to make it an outstanding cultivar. Breeders may cross this variety with a known source of the
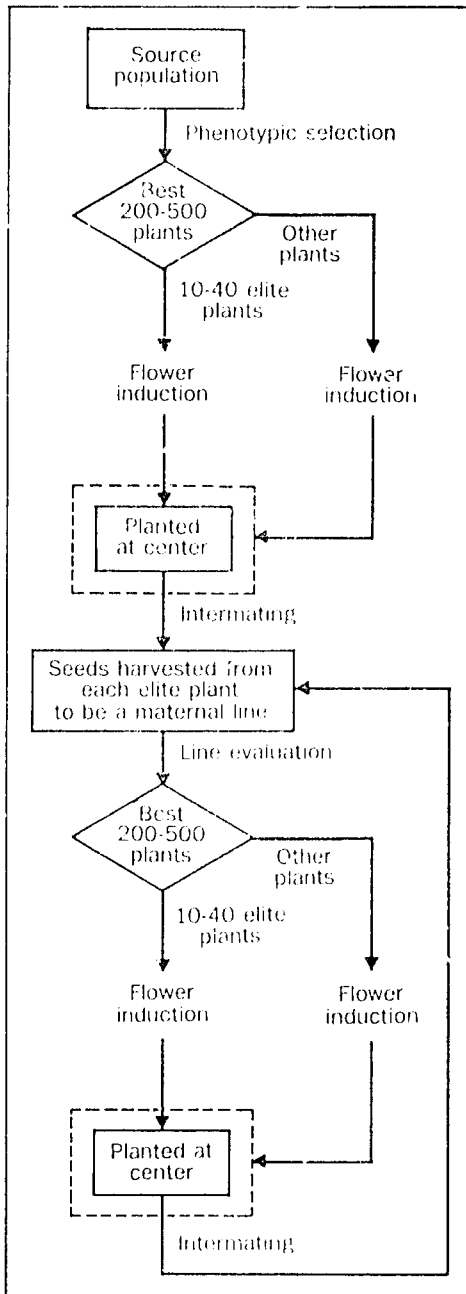
Figure 21. (left)
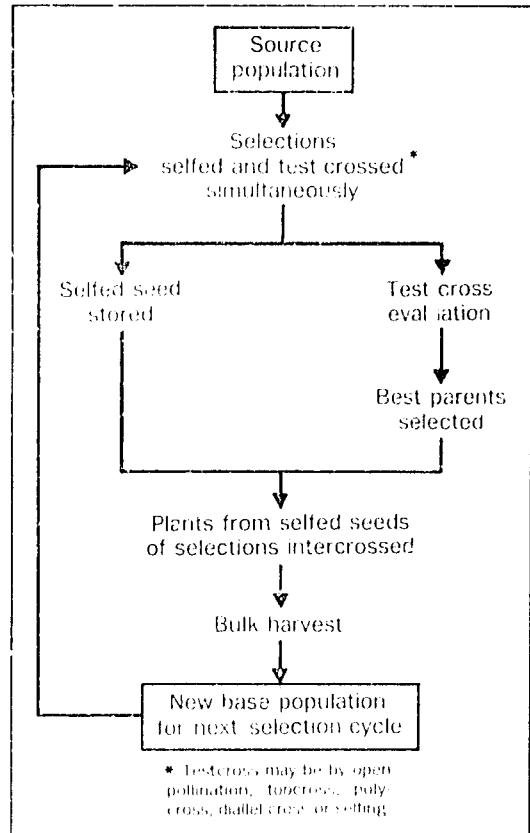Schematic diagram for procedures of maternal-line selection.

Figure 22. (above)
Schematic diagram for procedures of recurrent selection.

desired character to develop a new cultivar possessing that trait. Breeding techniques such as mass selection, family selection, or other methods using elaborate progeny tests may be applied, as the case may be, to obtain the improved type carrying the desired trait in fixed form and in combination with other good horticultural attributes. If the breeding program has a broad range of objectives and the variety in question already carries most of the desirable characters, the use of the backcross breeding method would be the most appropriate and effective.

In the backcross method, the F_1 generation of the cross between the variety lacking the desired character and the source or donor parent is crossed back to the former (referred to as

'recipient' or 'recurrent parent'). Backcrosses to the recurrent parent are then repeated for several generations, making sure that in each instance, the character under transfer is not lost.

The backcross method is simple if the desired trait is controlled by a single dominant gene. In each backcross generation, such a character will express itself and straight backcrossing may be carried out after discarding the backcross segregants which do not carry the gene. If the desired trait is controlled by a single recessive gene, it is necessary to proceed to the F_2 generation of each backcross so that the individuals possessing the desired gene can be identified. Alternatively, two generations of backcrossing may be interspersed with a cycle of backcross F_2 progeny tests before proceeding further. This is termed the 'blind backcross' method. In this method it is important to use a relatively large sample of plants in initiating every backcross generation in order to maximize the chance of keeping individuals carrying the desired gene in the sample.

The number of backcrosses to the recurrent parent depends upon the aim of the breeder. At one extreme, a sizable reconstitution of the recurrent genetic background may be desired. In this case the number of backcrosses should be no less than five and could be as high as ten. The final product is a variety that strongly resembles the recurrent parent but carries the missing desirable trait. At the other extreme, a certain amount of recombination between the donor and recurrent parents may be desired especially if the former has other desirable characters aside from the specific trait under transfer. A few backcrosses, possibly two to four, may then be followed by other selection methods to fix the desired gene in combination with the ideal horticultural types arising from the genetic recombination between the donor and recurrent parents. In this scheme the resemblance between the recurrent parent and the final backcross derivative will not be as strong as in the full backcross.

The advantages of the backcross method are as follows: it is predictable and repeatable; it is relatively rapid; previous gains are preserved intact; the program may be independent of the environment; and the evaluation of backcross derived varieties may not be essential.

Hybrid Breeding

When other systems of selection fail to stabilize or fix a desired character in the population, selfing is the ultimate choice among progeny testing methods. Its applicability as a breeding method by itself (line selection) is limited, however, because *Brassica* crops frequently suffer from strong inbreeding depression. In crops where selected plants may be vegetatively propagated, selfing is a very powerful tool to recognize heterozygotes carrying an undesirable recessive factor. The breeder can then fall back on the vegetatively maintained selection to reconstitute a new population for selection. Apart from its role as a powerful progeny testing tool, selfing has its most significant contribution in the development of uniform inbred lines for hybrid production. By virtue of having maintainable inbred lines, hybrid varieties can be reproduced time after time. Hybrid varieties in crucifers have several advantages. They are qualitatively and quantitatively better than standard open-pollinated varieties in terms of earliness, uniformity, and productivity (Fig. 23). With respect to disease resistance, it is far quicker to pyramid more genes into F_1 hybrids especially if resistances are governed by dominant genes. Through the selfing process, many undesirable recessive genes are eliminated from the progenies.

Important considerations in a hybrid program. The overwhelming advantages of F_1 hybrids over standard varieties in many cultivated crops, especially among outcrossing species, are often considered as compelling reasons to embark on hybrid breeding. However, breeders must take stock of the circumstances before finally settling on a decision to develop hybrids, instead of standard cultivars. These important circumstances are discussed here with occasional references to the Chinese cabbage improvement program at AVRDC.

Utilization of hybrid vigor: The dramatic improvement in the performance of hybrids is one of the most important, although not the only criterion in opting for F_1-hybrid
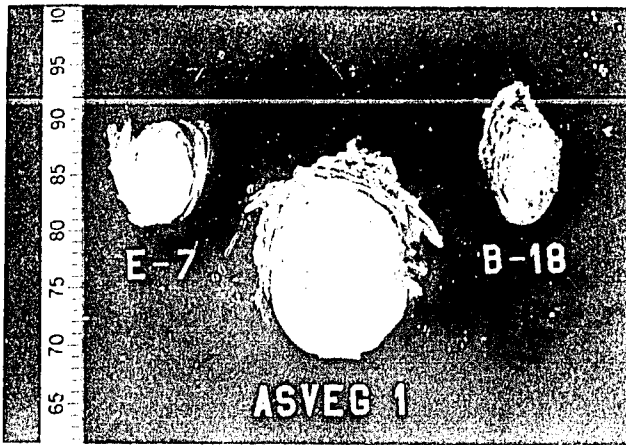
Figure 23.
Comparison of head size of an
F_1 hybrid (center) with that of
two parental lines.

development. In many crop species, hybrids often give higher yields, improved earliness, and uniformly better quality and disease resistance than standard cultivars. There are instances, however, when hybrids have been produced even without the above advantages as long as a high premium for other aspects of consumer acceptability exists. An often-cited example is the development of sweet corn hybrids in the United States where consumers are known to have a high regard for uniformity in size and general appearance of marketed ears.

The interest on hybrid development of Chinese cabbage at AVRDC was dictated, in large part, by the need to substantially increase the yield of heat-tolerant cultivars. Hybrid vigor in Chinese cabbage, about 20 to 50% better than parents, is of course well-known. Although heterosis was minimal among tropical varieties to warrant its immediate exploitation, appropriate genetic manipulations enabled its maximization and dramatically improved the performance of heat-tolerant Chinese cabbage hybrids (Opeña and Lo 1979, 1981).

Availability of mechanism for hybridity: A good portion of the cost of hybrid seeds results from the cost of seed production and maintenance of parental inbred stocks. Breeders must, therefore, take into account the presence of a suitable mechanism for hybridity so that hybrid seeds may be produced economically and released to growers at a reasonable price.

Hybridity mechanisms may come in the form of self-incompatibility, cytoplasmic male sterility, genic male sterility, dioecy or other systems (see Self-incompatibility, and Male Sterility, p. 19 and p. 32, respectively). If a suitable mechanism is absent or undeveloped, the decision to initiate a hybrid program must be weighed very carefully. Often, prolificacy in seed production becomes an important consideration as the hybrid seeds will have to be secured through manual means. Good examples of this case are abundant among the hybrid seed programs in self-pollinated crops like tomato, pepper, eggplant, etc. In some instances genic male sterility among these crops may be utilized to dispense with the need for emasculation.

Among crucifers, self-incompatibility is widespread and the question of a suitable scheme is hardly a moot issue. Nevertheless, the strength of recoverable S-alleles, in relation to the best available environment for seed production, should be considered.

Readiness of the market for hybrid seeds: Sometimes, the advantages of hybrids over standard cultivars are not sufficient guarantees for the acceptance of hybrid seeds. This situation is often met in the marginal areas of production where reliability, rather than maximum performance of varieties, is a virtue. Farmers in these areas generally save their own seeds from year to year. Open-pollinated varieties, or other genetically variable populations such as synthetic varieties, are comparatively more successful here than hybrid varieties.

Even in nonmarginal areas, the capability and willingness of growers to allocate extra input to meet the cost of seeds must be considered. If farmers usually save their own seeds for the

next season's production, it must be clearly demonstrated that the use of hybrid seeds can easily offset the additional costs of purchasing them time after time.

In the case of tropical Chinese cabbage in Taiwan, the overwhelming demand for hybrids was largely attributable to the attendant risks of growing Chinese cabbage during the hot, typhoon-plagued, monsoon season. By virtue of their uniform maturity and earliness, hybrid Chinese cabbage can be grown quickly and harvested collectively, thus, minimizing crop loss due to heavy rains and strong winds.

The acceptability of hybrid tropical Chinese cabbage in other tropical countries is often questioned mainly because the technology and infrastructure for hybrid seed production are not well-developed and that, even assuming acceptability, farmers may not be able to afford the extra cost of hybrid seeds. However, it can be argued that technology is transferable and that the additional seed cost may not be all too prohibitive because Chinese cabbage is small-seeded (1 g consists of approximately 300 seeds) and is usually grown only on a small scale.

Basic procedures in developing F_1 hybrids. Finding the best F_1 hybrid cultivars is not purely a matter of chance. There are systematic steps involved in hybrid development and these are discussed below. Again, the AVRDC hybrid breeding program for tropical Chinese cabbage is referred to sometimes as an illustration. Knowledge of the genetic bases for most, if not all, important characters desired in a hybrid is essential and, for simplicity, it is assumed that this information is generally available to the breeder.

Sources of inbred lines: Inbred lines may be derived from different sources, such as open-pollinated varieties, synthetic varieties, mass-selected populations, recurrently selected populations, populations originating from intervarietal crosses, etc. The choice of possible sources is wide; yet when the breeding objectives are taken into account, there may be only a few viable alternatives. Often, these are locally adapted, open-pollinated cultivars of proven worth which carry a number, if not all, of the important characters of interest to the breeder. This is common, especially at the beginning of a hybrid program.

In more developed programs, inbred lines are often sought from genetically improved populations such as those arising from mass selection and recurrent selection. Individual plants are selfed through successive generations until homozygosity is reached, and the inbreds are stable for morphological and physiological characteristics. For example, the inbred lines used in the development of tropical Chinese cabbage hybrids at AVRDC originated from genetically variable populations that were improved through mass selection for heat tolerance and disease resistance after they were initially derived from intervarietal crosses (see Genetical and breeding aspects of heat tolerance, p 36.).

Selection for combining ability and other horticultural traits: Not all genotypes derived from a source population will possess the desired 'nicking' ability or prepotency to produce superior offsprings in combination with other genotypes. It is, therefore, essential to identify the lines with good combining ability. This could be done early in the inbreeding process, thus, allowing efforts to be concentrated on the few lines with superior combining ability. Alternatively, the breeder may attempt first to stabilize the lines for certain important traits, including selection for strong self-incompatibility, before they are tested for combining ability.

Each of the above-mentioned methods has its own merits and limitations. Early generation testing of combining ability may save time and resources as further inbreeding is restricted only to the superior combining lines. However, the genotypes under test are still highly heterozygous and this often necessitates the use of large progeny samples in order to derive reasonably accurate inferences about their combining ability. Otherwise, the combining ability in the later generation may deviate greatly from earlier estimations. With genetically stable lines, this problem is substantially simplified but the time and resources spent in the inbreeding of lines, with otherwise inferior prepotency, may be prohibitive.

Assuming that the early generation testing scheme is adopted, the lines would be first tested for general combining ability (GCA). The tester stock in this case would be a genetically variable

population (e.g. open-pollinated variety) and the type of test is called the topcross method. The topcross progenies are then evaluated, preferably in replicated fashion, to determine the superior combining selections. Only the best combining lines are propagated further by selfing. These few remaining lines are then tested in specific combinations, either by crossing them to a common inbred tester stock or among each other, especially if the number of all possible combinations is manageable. This specific combining ability (SCA) attempts to identify the best specific combinations. The SCA test may be applied to all possible crosses among the selected GCA lines accompanying the subsequent fixation procedures for two to four generations (Ito 1954).

Since the relative importance of GCA and SCA may vary among characters, it is more efficient to emphasize the traits for which GCA is important in the early generation and then pay attention later to those traits where SCA has more relative importance. GCA is important for characters such as leaf number and maturity but not for unit leaf and midrib characters (Yoon et al. 1982). Among midrib characters, narrow-sense heritability was found to be high for length and area and low for width and thickness in a diallel study among temperate lines.

If progeny tests are to be applied after the fixation of desirable horticultural traits and self-incompatibility, a preselection for general combining ability may be carried out initially to reduce the number of lines to manageable levels. Again, lines would be topcrossed to a genetically variable stock following which, the topcross progenies would be evaluated in a replicated fashion to select the superior combining lines. A possible variation to topcross might be to allow all inbred lines to mate at random in isolated plots. Open-pollinated seeds of each line may then be harvested separately and evaluated for general combining ability through a polycross test. The superior combiners from the GCA test are then evaluated for specific combining ability by crossing them in all possible combinations or with common inbred testers. The best specific combinations for the traits desired in hybrids are then earmarked for release to growers after sufficient performance and seed-production trials.

The method of GCA test by topcrossing, followed by the SCA test in all possible combinations accompanying the fixation procedure for two to four generations (Ito 1954) or a modification of it in which as many crosses as possible are applied among lines at the line-selection stage, followed by the SCA test on all possible combinations among selected lines (Haruta 1957) are both time-consuming and laborious. Park and Hyun (1981) proposed a simplified testcross in which stable parental lines are grouped by some predetermined visual criteria and the combining ability is carried out on any new inbred belonging to a group by crossing it with a fixed tester of another group and vice versa. Although this scheme is more time- and resource-efficient, it has the possible limitation that the sorting of lines according to some predetermined criteria may accelerate the narrowing of genetic diversity among breeding lines. Such a diminution of genetic variability may lead to the loss of intermediate lines carrying important genes.

Production of hybrid seeds: A hybrid combination with good potential for commercial release should be adequately tested for its performance, not only in production fields but also in seed production lots. For this purpose parent lines are planted alternately (other schemes may be used depending upon the circumstances) in isolated plots to produce the hybrid seeds for such trials. It is presumed that by this stage, only parent lines that have proved to be sufficiently uniform are used. The hybrid seeds from this lot are then evaluated for the important characters that new cultivars must possess. At the same time, the hybrid seed lots are tested for sibbing rate. Sibbing rates above the commercially acceptable standard reduce the potential value of the hybrids. However, decisions to discard or promote the hybrids must be based on a thorough analysis of the genetic and environmental factors that may have influenced the seed production.

Multiplication of parental lines: The parent lines of hybrids that show good potential for commercial release in the early stages of the evaluation trials should be multiplied in preparation for large-scale hybrid seed production. This will involve either massive bud pollination or other alternative schemes to overcome self-incompatibility (see Maintenance and multiplication

of SI parents, p. 61). In such a multiplication, biological disturbances arising from bud pollination and inbreeding, e.g. heteroploidy and other abnormalities (Iizuka 1960), should be noted. The eventual use of these seeds in hybrid seed production must be accompanied by careful roguing of off-types in seed beds and in the seed production field to insure that the hybrid seed lot is not contaminated.

Seed Production

Environment

Environmental factors are important in the production of Chinese cabbage seed. The same climatic factors which influence the cultivation of Chinese cabbage as a market vegetable also act on seed production. The favorable factors are as follows: low atmospheric humidity; minimal rainfall; adequate irrigation; and optimum temperature. Furthermore, suitable daylength, good soil, adequate fertilization, and optimum sowing or planting time are essential. The role of bees in pollination is also important. Any biological response elicited by these factors will invariably affect seed production. Plant characters such as days to flowering, days to seed ripening, number of branches, plant height, raceme length, silique number, seed number, and seed yield are influenced by environmental conditions such as altitude, planting date, irrigation and fertilizers, spacing, plant density, and temperature.

To produce disease-free and high-quality Chinese cabbage seed, it is advisable to select a dry climate or at least a season with a low air humidity, especially during the seed-ripening and harvesting stages. A dry climate discourages disease development and favors seed drying. When humidity is high, as in a humid tropical climate, special arrangements have to be made for artificial drying and seed storage.

During the cultivation period for seed production, the control of pests and diseases has to be given careful attention. The control of both of these factors in the humid tropics makes the production of good quality seeds rather expensive.

The soil quality has also played a part in the development of seed production areas; generally only those loam soils with pH 6.0 to 7.5 and a relatively high water-holding capacity are most suited. Soil conditions are especially important when Chinese cabbage plants are lifted after selection and replanted or young plants are transplanted into their final seeding quarters. The nutrient status of soils can be modified by application of appropriate macro- and micronutrients, but those soils with a satisfactory nutrient status and a satisfactory cation exchange capacity are most useful.

The daylength reaction is very important for seed production of Chinese cabbage. Chinese cabbage is a long-day plant and does not normally flower in the lowland tropics because the change in photoperiod in the tropics is minimal (Fig. 24). However, it has been proven that low temperature can replace the photoperiodic requirement of Chinese cabbage to some extent; thus, it can bolt and set seed when cultivated at high elevations of the tropics, where the temperature is cool.

Temperate Chinese cabbage normally requires a cold period, from a few days to several weeks, below 5° to 12°C, for flower induction and development. The seed-to-seed or head-to-seed method of temperate Chinese cabbage seed production, using natural cold periods, is not feasible in the tropics, even at altitudes between 800 and 1200 m (Fig. 25). Chinese cabbage must be either vernalized at the seedling stage or their mature plants uprooted for vernalization in a cold storage, in order to induce flowering.

Tropical Chinese cabbage varieties, on the other hand, can produce seeds without requiring very low temperatures. The vernalization requirement of these tropical types can be fulfilled at rather high temperatures, i.e. near or below 20°C for a period of time, supplemented with artificially extended photoperiod, can be effective; the temperatures at higher altitudes in the tropics can usually satisfy this requirement (Fig. 25). The establishment of precise temperature and photoperiod requirements for each tropical variety at a specific location usually requires study.
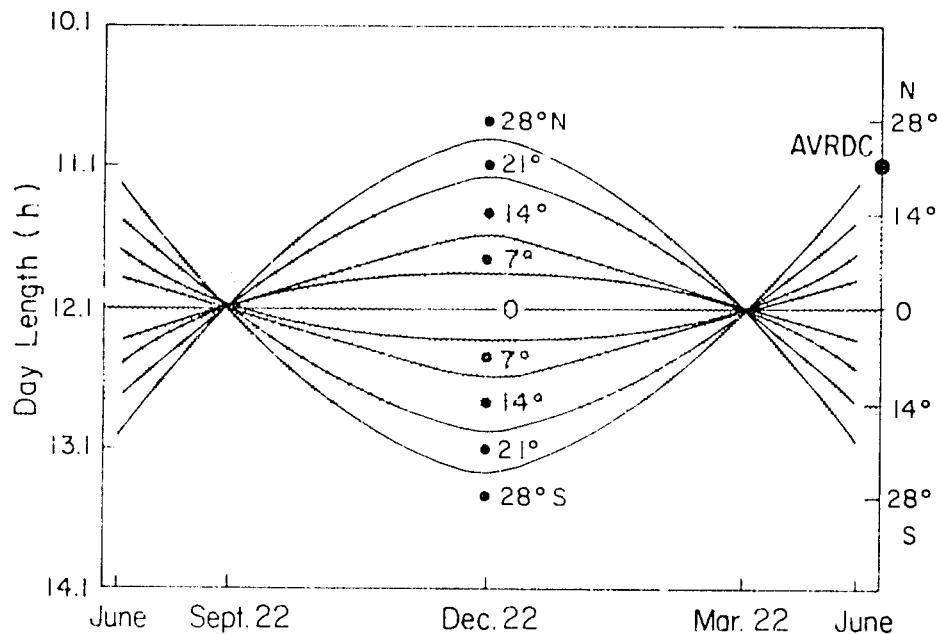
Figure 24. (above)
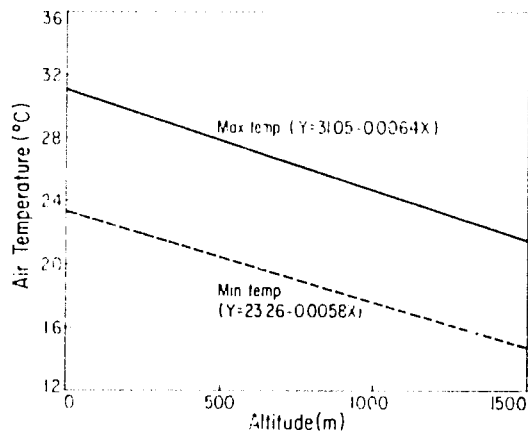Daylength variation at differen
latitudes.

Figure 25.
Relationships between altitude and
temperatures in the tropics.

Based on the above criteria of climate, soil, and other ecological parameters, seed production of Chinese cabbage in the tropics would appear to be difficult. However, there are already areas in the tropics where Chinese cabbage seed production has been proven feasible through appropriate practices like proper site selection and other improved techniques; notable examples include the Philippines, Taiwan, and Thailand. This type of development is stimulated by the advancement of technology generated by AVRDC, as well as by the national programs, and the economic prospects for private seed companies.

Production Procedures

Head-to-seed method. This method is usually employed in the breeding process to develop parental lines and examine the best hybrid combinations, or for breeder and foundation seed production of open-pollinated varieties (Fig. 26). The plants for seed production are grown until head formation under the same cultivation method as commercial culture in order to select the
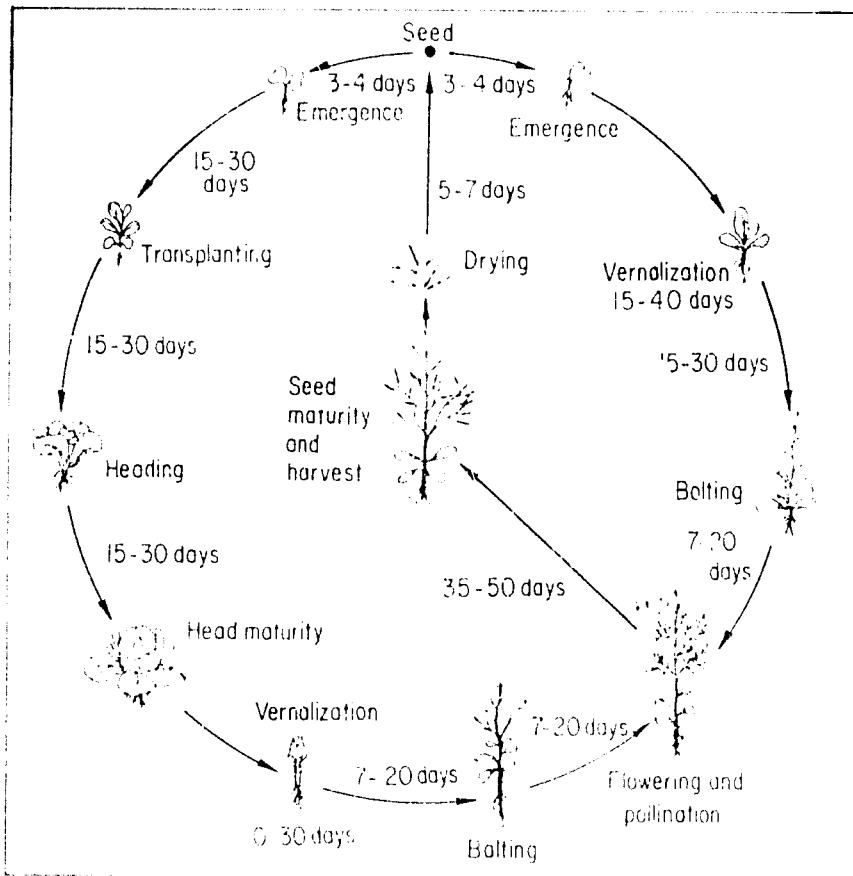
Figure 26. Diagram of two different generation cycles (head-to-seed vs. seed-to-seed) in relation to breeding and seed production of Chinese cabbage.

best plants. This method is not used to produce large amounts of seed because the seed plant becomes aged, nor is it regularly practiced for the breeder's seed production of inbred parental lines because the seeds are already genetically pure.

Plant production: Sowing dates depend on the local environment, custom, and experience with specific varieties. Sowing should be timed so that the variety receives sufficient vernalization, and that the plants flower and seed under suitable climatic conditions. Sowing in the flat at 5.5 cm × 6.0 cm spacing, and two to three seeds per spot is recommended. In the case of simple mass selection, 20-50 times the number of needed plants are usually grown to secure the idealized number of selected mother plants.

Young plants from the seed flats are transplanted when they have reached the sixth to eighth leaf stage. Obvious off-types in foliage characters, blind plants, and plants showing signs of serious diseases are rogued out at this stage.

The spacing at transplanting depends on the vigor of the variety. The distance between furrows bounding each bed is usually about 1.5 m. Each bed consists of two rows with plants within and between the rows spaced 50 cm apart, respectively. A furrow depth of 20 cm during the dry season or 30 cm during the wet season is employed.

In case of parental line selection, the mature head is checked for characters such as shape, relative size, firmness, and maturity according to the standard characteristics of the variety.

Leaf characters such as color, wrinkle, and pubescence are also examined. The desired plants are then selected; the rate of selection ranges from 2% to 5% of the population.

Handling of selected mother plants: In case of parental line selection, the selected plants are carefully dug out to remove soil from the root while keeping as much of the root system intact as possible. The plants are then stripped of their lower and outer loose leaves, transplanted into the growing pots (ID 25-30 cm) with sterile medium, and transferred into the nethouse or cold room which is cooled to 5 to 10 C with 80% RH for vernalization. The upper portion of the heads are then cut horizontally into one-half to one-third segments and then cut vertically to form a pyramid around the heart of the head, avoiding injury to the growing point and the inner yellow leaves (Fig. 27). Details of other methods of cutting heads are described by McCaffery et al. (1986). In about a week, the lower leaves will begin to absorce. Removal of the leaves that have absorced is done stepwise from the basal to the upper leaves, about three to four times for a period of a few weeks, until the inner yellow leaves start growing and turning green. The exposed parts where the leaves have been removed should be immediately dusted with protectants such as sulfur powder. Plants are often watered by the drip irrigation method to avoid excessive moisture. After seed stalks develop, they are loosely tied to two to three supporting sticks to protect the fruiting branches from lodging. Sticks of about 1.5 m height are driven into the pots, and the twine is strung between the sticks to keep the fruiting branches in an upright position.
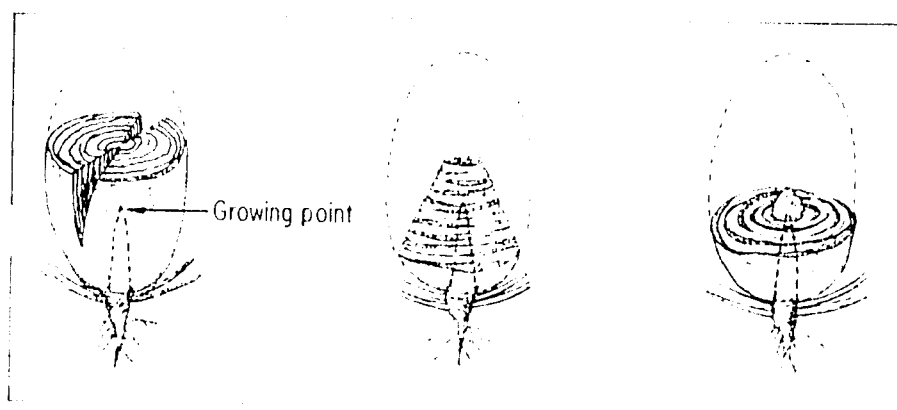

Figure 27. Different methods of cutting open the Chinese cabbage head for seed production.

Seed-to-seed method. Commercial seed production usually adopts the seed-to-seed method by which seeds are produced from seed plants which are induced to bolt and seed without passing through the heading stage (Fig. 26). The advantages of this method are efficient land utilization, on both a time and space scale, and higher seed yield per unit area because of lower softrot incidence. Since strict selection of seed plants for varietal characteristics cannot be applied in this method, the breeder seeds for multiplication should be genetically pure beforehand.

Breeder seeds are either sown directly in the field with several seeds per hill in case a sufficient amount of breeder seeds is available, or in the nursery beds to be transplanted later in the field in order to efficiently utilize the breeder seeds. The direct sowing method is less intensive and suited for large-scale seed production. The transplanting method provides an opportunity to detect certain off-type plants based on color, shape, and wrinkle of seedling leaves. The time of setting seedlings in the transplanting method directly influences the performance of seed production.

Spacings in the field for both methods are 60 to 80 cm between rows, and 20 to 40 cm within rows. The final spacing depends on the vigor and branching habit of the variety but a

useful criterion is to ensure that plant density is as high as possible while allowing sufficient space for inspectors to examine the crop or for easy intercultivation.

Seeds are sown in both methods at a time when a sufficiently low temperature is still available for vernalization. However, germinating seeds of Chinese cabbage in the transplanting method can be vernalized. GA₃ at 200 ppm applied two weeks after transplanting to four-week-old seedlings compensated for the inadequate vernalization of Chinese cabbage, and increased seed yield in the tropics (Piluek 1985). Careful practices in seed production must be followed to overcome the low survival rate under hot, humid conditions of the tropics, and the poor vegetative growth of plants due to early flowering.

Field management and fertilization. The AVRDC International Cooperator's Guide (Yoon et al. 1987) provides general guidelines on the recommended cultural practices in growing Chinese cabbage for head yield. This guide may be roughly followed in the management of Chinese cabbage plants for seed production.

Selection of sites with sunny conditions, fertile soil with good drainage, and easy access to water supply are suggested. The faster the effect of nitrogen fertilizer in the soil dissipates, the earlier will be the time of flowering and of seed harvests, and the lesser will be the amount of seed produced. If nitrogen is supplied for too long a period, harvest time becomes late, the plant falls over, and damage by diseases and insects is increased. Since supplying a sufficient amount of nitrogen at the initial stage of flowering is most effective to increase seed yield, side-dressing at this period is recommendable. Adequate potassium has to be supplied also at the early stage of flowering. Furthermore, the boron deficiency symptom, which sometimes occurs, tends to cause a decrease in seed production.

Split application of 150 kg nitrogen, one-half applied prior to planting (basal) and the other half side-dressed at two weeks after planting, is recommended for the seed production of artificially vernalized Chinese cabbage plants (Hou 1981). In addition to the recommended fertilization rate, additional side-dressings of N, P₂O₅ and K₂O at the rate of 30, 30 and 20 kg/ha, respectively, at bolting time, and 15, 0 and 10 kg/ha, respectively, at mid-flowering stage, are suggested. Borax at approximately 10 kg/ha is applied beforehand to the seed production field where boron deficiency symptoms may occur.

If Chinese cabbage seed production is conducted in a region where precipitation is low, additional water supplied through irrigation may be highly desirable. Drought stress frequently occurs under tropical or arid conditions and can increase the difficulty of confirming some foliage and head characters during roguing. Timely irrigation from heading to midflowering stages, or at the time of side-dressings are essential for Chinese cabbage seed production. However, irrigation should be given from the seed stage to the harvesting stage in order to avoid excessive vegetative growth.

Decapitation of newly bolted plants stimulates the development of axillary buds which later can develop into side branches for increased seed yield (Thomas 1983). The ideal number of branches ranges from 10 to 20 for tropical or early-maturing varieties. Occasionally the terminal tips of the secondary and tertiary flowering branches are pruned to avoid further branching and to improve seed yield and quality.

Disease and pest control. Mosaic virus, bacterial softrot, downy mildew, and Sclerotinia rot are important diseases which affect seed production.

Aphids transmit mosaic virus to Chinese cabbage plants. Throughout the growth stages, the overlapping leaves provide an excellent shelter for aphids. Thus, their control is necessary from the seedling stage in order to minimize the damage by viruses.

Insecticidal treatment should be initiated as soon as more than 1% to 2% of the plants in the field are infested with aphids. Prothiophos (0.5 kg a.i./ha) or pirimicarb (0.5 kg a.i./ha) provide effective coverage when sprayed on the undersides of leaves. Viruses cannot survive in the absence of a living host; they depend for survival on perennial and annual plants. Thus,

planting next to an old virus-infected crucifer field should be avoided, and weeds such as wild mustard should be controlled.

No chemical is effective for the control of bacterial softrot. Fields with good drainage or low water table may prevent disease incidence. The use of high beds, sparse population density, straw mulch, and the reduction of insect and mechanical injury can also minimize disease incidence (AVRDC 1985, Fritz and Honma 1987). Softrot- and virus-infected plants must be destroyed.

Fungicide treatment is needed when downy mildew occurs early in crop development. Spraying with Dithane M-45 33% (500 ×) or Ridomil MZ 58% (400 ×) once a week throughout the growing period until early flowering is recommended.

To reduce the incidence of Sclerotinia rot, deep plowing, keeping bed surfaces dry with careful irrigation, and removing weeds and infected crop residues from the field may be helpful. Spraying a few times with Ronilan (200 ×) at one-week intervals also provides a good control of Sclerotinia rot. Fungicidal sprays for downy mildew and Sclerotinia rot do not have any significant harmful effect on honeybees.

Beside aphids, diamondback moth (*Plutella xylostella*) is another serious pest problem that deserves careful attention. After emerging from eggs, larvae of diamondback moth feed mostly on the underside of outer leaves, chewing out small holes or at the growing points of younger plants. Control of this pest, therefore, should be based on the number of larvae. Insecticide sprays to control aphids and diamondback moth are harmful to honeybees. Therefore, it is recommended to secure adequate control of these pests from the early stage of growth and cease spraying 7-10 days before the bees are released. Microbial insecticide *Bacillus thuringiensis* is effective in controlling diamondback moth without considerable harm to honeybees, and may be used extensively after the introduction of honeybees. In the seedling stage, spray teflubenzuron at the rate of 37.5 g a.i./ha. This treatment will reduce pest population buildup. Incandescent lights kept lit at night, and pans containing water which are placed underneath the lights in the cage are effective in trapping the adult insects and can be used as a supplementary control measure.

Harvesting and storage.   The Chinese cabbage siliques ripen in more or less the same sequence in which the flowers open. As the seeds ripen, the siliques start to dry out. Dry Chinese cabbage siliques have a strong tendency to shatter. The loss of seed from shattered siliques due to impact between siliques and other plant parts and machinery, wind, and internal stresses brought about by thermal effects and by drying can be a major problem in seed production. This loss may occur before or during the harvest process (Fig. 28). One method used to reduce the loss is by windrowing of the crop just prior to ripening. Shattering resistance associated with lower levels of lignification of the silique structure has been found in *Brassica* sp., and has been recommended for incorporation in the breeding program (Kadkol et al. 1984).

When a noticeable proportion of the silique have turned orange-brown, at which time the seeds will not crush or split when rubbed between the hands, the seed crop should be at an
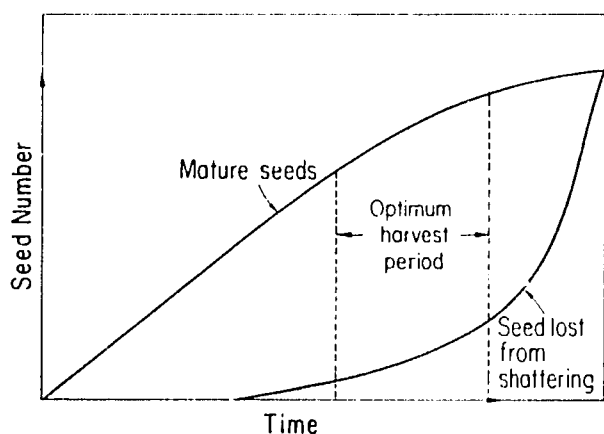
Figure 28.
Schematic representation of the interaction of seed maturity, potential yield and loss from shattering.

appropriate stage for harvesting. Many seed producers prefer to cut the ripening stalks and continue to dry them in a sunny place. When 80% of the siliques have turned orange-brown, the plants can be severed at the bottom. Several plants may be grouped in bundles and then hanged on a pole for several days under the sun for after-ripening and drying.

About one week later, when all of the siliques are completely dried, the siliques are then threshed on top of plastic sheets placed on level ground. The immature seeds, which cannot be dislodged from the siliques and branch pieces, are cleaned out by standard mesh sieves or winnower. Sequential sievings with 3.0 mm and 1.3 mm openings enable the removal of most chaff particles. The seeds are then dried again on the plastic sheet under the sun.

The moisture content of fully dried seeds is about 12%. Since the specific density of seeds is higher than water, the healthy seeds should sink in the water. The average 1000-seed weight of Chinese cabbage is from 2.5 to 3.5 g; however, average seed size and weight usually decrease with the increasing order of flowering branch (Jiang 1981).

Properly dried and cool-stored seeds can retain good viability for three to four years since seed aging, under these conditions, advances very slowly. The main requirement for seed storage in a hot, humid climate is to prevent the well-dried seed from regaining moisture from the atmosphere. Bagged seed in the refrigeration unit should be adequate to counteract the storage problems.

Hybrid Seed Production

Maintenance and multiplication of SI parents.   In the case of F_1 hybrid seed production, both parental lines should be self-incompatible but cross-compatible (Fig. 29). The reproducibility of hybrids depends absolutely on the presence of parental inbred lines that can be multiplied easily and economically. As the parental inbred lines are self-incompatible, their maintenance is achieved mainly through the exploitation of the phenomenon of 'juvenile compatibility' (Kakizaki 1930). The self-incompatibility mechanism is not operative at the bud stage, and thus, selfed seeds can be produced if pollinated two to three days before blooming. Bud pollination has been used as a routine technique to maintain self-incompatible inbreds. Multiplication of parental stocks can be done by artificial bud pollination in the nethouse. Bud pollination, however, is always laborious and expensive. For this reason, there have been attempts to find commercially feasible ways to overcome self-incompatibility. Among alternative means, salt and CO_2 treatments seem to be the most promising.

Chinese cabbage is predominantly cross-pollinated although some self-pollination can occur. Caution is necessary to prevent possible contamination. Most authorities agree that an isolation
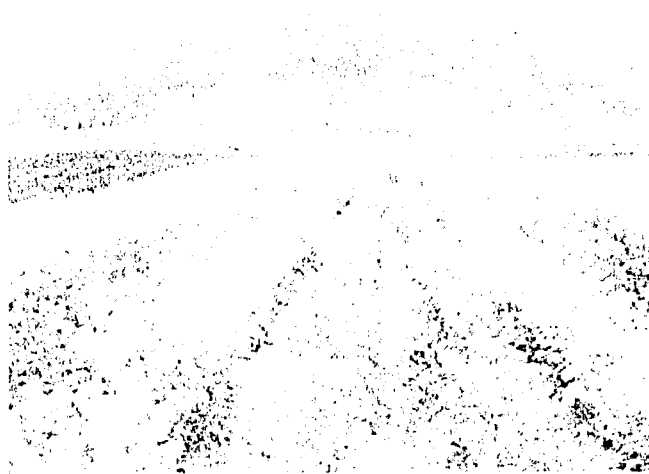

Figure 29.
Seed production field of F_1 hybrid Chinese cabbage showing two parental lines with distinct plant types. These parent lines are self-incompatible but cross-compatible with each other.

distance of at least 1000 m between different subspecies of *B. campestris* or varieties of *B. campestris* subsp. *pekinensis*, or at least 200 m between different genomes of *Brassica* is necessary.

Pollen may be bulked from a large number of plants of a parental inbred line to pollinate all plants within that line. In this manner drastic inbreeding depression such as would happen with individual plant selfing may be easily avoided.

It is generally recommended that a sufficient quantity of parental stock seeds be produced to meet the requirements for succeeding years. In this manner yearly regeneration of stock seeds may be avoided. This cost-saving practice also serves to slow down inbreeding depression provided the seeds are stored under proper conditions.

Intensive roguing must be exercised in the seedling nursery to remove all off-types for any horticultural or reproductive trait in the parental lines. This serves to insure the integrity of the hybrid.

Surmounting the incompatibility barrier.   Numerous environmental and physiological factors affecting self-incompatibility have been applied successfully to overcome the incompatibility barrier (see Modifications of self-incompatibility expression, p. 23) and enable the production of seeds from self-incompatible pollinations. Most of the important and practical methods are discussed here.

Bud pollination: This is by far the most commonly used method to overcome incompatibility. It is usually performed by applying mature pollen from the same plant or bulked pollen of the same line to pistils a few days, usually two to four days, before anthesis. The following procedures are generally observed:

1. Flowering plants of the self-incompatible inbreds are usually kept in isolation cages or nethouses to avoid contamination. Flowers that may have been possibly contaminated before isolating the plants should be removed.
2. Bud pollination involves opening of the young bud, usually the eighth to tenth bud from the very bottom of the raceme, to expose the stigma for pollination. Bud pollination is usually carried out between 7 a.m. to 11 a.m. Precautions should be taken to avoid damaging floral buds or pedicels while conducting pollination. Since open flowers generally will not set upon selfing, they are used mainly as a pollen source. Very immature buds in the branch are not pollinated, instead they are left for future pollination once they reach the appropriate age.
3. Bud pollination is carried out in succession until the seed set potential of the plants begins to decrease. At this time further bud pollination, even of the newly emerging branches, is considered no longer worth the effort. Many young buds will begin to abort prematurely. This condition is reached sooner in potted plants than those planted directly on the soil and must obviously relate to inadequate nutrition.
4. If the isolation cage or nethouse is insect-proof, no bagging is required.
5. Inbred lines that show drastic inbreeding depression should be sib-pollinated rather than continuously selfed. Sibbing is a milder form of inbreeding compared to selfing and can slow down further deterioration in the vigor of such lines. Bud pollination is also recommended for sibbing since sibs may share common self-incompatibility allele(s), especially in an S-allele homozygous line. In practice, after obtaining a practical level of homogeneity in the inbred lines, further depression has to be prevented through intermating of individuals within the same line.
6. In order to avoid inadvertent contamination, spontaneous genetic changes, and rapid decline of vigor among inbred lines, the frequency of maintaining parental inbred lines should be minimized. In this case production of large amounts of inbred seeds may be done during the early years of their involvement in hybrid production and the bulked seeds stored under long-term storage conditions. The required amounts for hybrid seed production may then be withdrawn year after year until exhaustion of the seed supply at which time another round of large-scale bud pollination may be carried out again if necessary.

A substantial portion of the cost of hybrid seeds traces back to the cost of multiplying the parental inbreds through bud pollination. For this reason there has been considerable interest in finding other commercially feasible ways to overcome self-incompatibility (Ito 1981).

Carbon dioxide (CO_2) treatment: The carbon dioxide treatment is effective in overcoming self-incompatibility in *Brassica*, and has been successfully applied to breeding in *B. campestris* (Dhaliwal et al. 1981), *B. campestris* subsp. *pekinensis* (Lee 1979, 1981, Tao and Yang 1986), and *B. napus* (Thompson 1978). It greatly reduces the production cost of selfed seeds as compared to bud pollination in Chinese cabbage (Lee 1979). CO_2 treatment, in combination with honeybee pollination, can reduce the cost of production to less than half that of bud pollination. Application of 3% CO_2 in the air for at least two hours beginning two hours after self-pollination of open flowers is the most reliable and most economical treatment (Fig. 30). However, differential response to CO_2 has been observed among self-incompatible strains. Generally, strongly self-incompatible lines tended to have lower fertility under CO_2 treatment than weakly incompatible lines (Ito 1981), and the genotypes with more seed set on bud selfing tend to be more responsive to CO_2 treatment (Lee and Shin 1985). However, the breakdown response to CO_2 treatment was more likely a function of specific genotype response rather than due to the strength of self-incompatibility itself (Lee 1981).
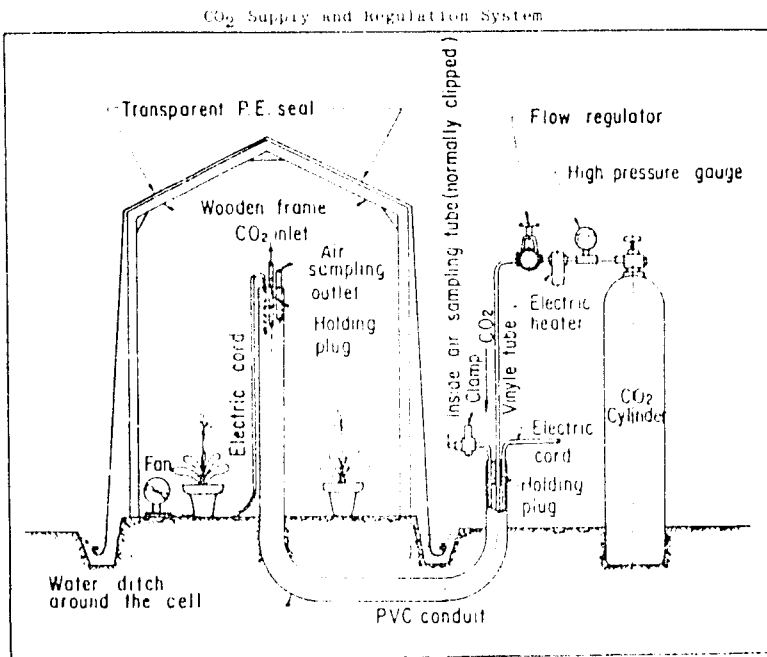
Figure 30.
Schematic diagram
of CO_2 treatment.

Salt treatment: Recently, it was found that 2% to 3% NaCl solution was effective in overcoming self-incompatibility of Chinese cabbage and other *Brassica* crops when sprayed to the flowers at one-half to one hour after pollination (Tao and Yang 1986).

The salt treatment method (Fig. 31) is the simplest and most effective among the proposed alternative means to increase self-incompatible inbred lines. This method seems less affected by genotype or level of self-incompatibility than the CO_2 treatment method. If combined with bee pollination in the isolation cage, silique and seed set rate is generally much higher than the CO_2 treatment method and comparable to bud pollination.

Although this method is inexpensive and simple, the optimum concentration and time of application may differ between plants and growing conditions.

Figure 31.
Effect of NaCl spray on selfed silique-setting on a self-incompatible Chinese cabbage line.

Other methods: Numerous other methods have been suggested to surmount the incompatibility barrier, e.g. delayed pollination, irradiation, chemical treatments, electric-aided pollination, and steel brush pollination. These methods usually involve elaborate techniques; thus, they may be applicable in physiological or genetic studies but are not recommended for breeding and seed production purposes.

Ideally, the most favorable technique for the breeder would be to massively propagate the parental lines in isolation under thermal conditions which promote self-fertilization of plants having the same S alleles. On the other hand, commercial hybrid seed production would be done under different thermal conditions, which will assure strict incompatibility. Unfortunately, this goal can be achieved in very rare cases only, since there seems to be a strong positive correlation between the breakdown of incompatibility of an S-allele at high temperature and the general incompatibility weakness of this allele. Thus, in *Brassica* the S-alleles which show strict incompatibility are very slightly (if at all) sensitive to thermal conditions, while those which are more apt to become pseudo-compatible lose their incompatibility activity at high temperatures. Nevertheless, appropriate breeding and selection may overcome this undesirable correlation.

Insect factors in hybridization. Three different insects, i.e. the honeybee, blowfly and drone fly, have been used as pollinators in caged or bagged *Brassica* flowers.

Honeybees are the best pollinator in large-scale seed production, although they do show a tendency to self- or sib-pollinate the parental inbred lines. Colonies of 3,000 bees are effective for *Brassica* pollination (Pearson 1932); small colonies made up of two standard combs are used in pollination of Chinese cabbage in small cages at AVRDC. Cages with little space over the canopy of the crops greatly encourage foraging on the flowers (Ellis et al. 1981). Prior to release for new pollination work, bee boxes have to be closed for a certain period of time to remove any possible pollen contamination. This period varies from one night to two full days in the AVRDC Chinese cabbage hybrid program.

In commercial seed production, the beneficial effect of honeybees in the open field is to provide stigmas with ample amounts of pollen and to maximize hybridity in the resulting seed lots through their thorough foraging of the flowering plants.

On a smaller scale, blowflies (*Calliphora* sp.) are known to be more convenient to use than honeybees. These are locally available in many places and can be reared using beef lungs as the larval food. They can be used in cellophane bags (Smith and Mee 1984), and small cages (Smith and Jackson 1976).

The drone fly (*Eristalis* sp.) can be used in cellophane bags, as well as in the open field, as an alternative to the blowfly and honeybee. However, its natural distribution is confined to the temperate zones, and its mortality increases with increasing temperature and insecticide application.

Generally, the above insects are highly sensitive to most chemical insecticides. However, spraying with a microbial insecticide such as *Bacillus thuringiensis* does not harm the honeybee and is still effective in controlling diamondback moth and other lepidopterous insects. Insecticides such as Kelthane, Nicotine and Neotran are known to be relatively nontoxic to honeybees. Nicotine fumigation kills aphids without great harm to blowflies. Demeton-S-methyl, applied in a water solution onto the surface of the compost in which the plants are grown, is taken up by the roots. This will also effectively kill aphids without serious harm to blowflies (Smith and Jackson 1976).

A low level of insect damage may have to be accepted since there seems to be no insecticide that is completely harmless to the insect pollinators. Good insect control before the release of insect pollinators is very important. No insecticide should be sprayed for a period of one week or longer prior to the release of pollinators; otherwise the residual toxicity of the insecticides could significantly decrease the pollinator population.

General procedures for commercial hybrid seed production.   Commercial F₁ hybrid seeds are produced in isolation during the optimum season for seed production. A simplified sequence of operations is as follows:

1. Sow parental lines on seedbeds or any appropriate medium, e.g. plastic pots, Jiffy pots, etc.
2. Where natural temperature is not low enough to vernalize the plants, seeds are sown in petri dishes lined with filter paper previously moistened with distilled water. Petri dishes are then incubated at 24 C for two days and vernalized at 5 C for two to six weeks, depending on the genotype. Seeds and all other materials should be disinfected in advance. The petri dishes have to be watered periodically during the incubation period. Provision of light and nutrients during the incubation period can increase the survival rate of seedlings when pricked. Dim light and watering with Hoagland solution are adequate for these purposes. When the treatment period is over, the petri dishes are taken out of the cold room and placed in a lighted room (ordinary room temperature) for about half a day before pricking. After pricking, seedlings should be kept under partial shade until fully recovered.
3. Transplant seedlings to the field at an appropriate time, depending upon the growth of the seedlings. Normally, this would be about three to four weeks after sowing or pricking. The inbred lines may be arranged alternately or according to some prescribed ratios. Lines should be properly labeled as to their identity.
4. Lines will begin to bolt and flower after some time. This period to bolting and flowering will vary with genotypes and environment. Assuming that lines with similar bolting requirements were used, they should ideally flower at the same time. Ordinarily, wild bee populations and other insects are sufficient for effective pollination. If deemed inadequate, artificially raised honeybees may be placed in the isolated production plot for better seed set.
5. With effective bee foraging, the pollination period should be completed in about three to four weeks. Under open field conditions, insect pollinators will continue their activity as long as fresh flowers are available. If resources permit, such late pollinations, which may have occurred when one or the other line has essentially finished flowering, should be removed. These late seeders often contain a high proportion of sibs (selfs).
6. At harvest, individual lines may be harvested separately and blended according to prescribed proportions, or bulk-harvested in the field if this is found appropriate. If the hybrid has only one parent with strong incompatibility, harvest only the seeds from this line and discard those produced by the weakly incompatible line. However, this scheme is uneconomical and should be pursued only under special circumstances. In this case, it is recommended to discard all the rows with the weakly incompatible pollen source at the end of flowering of the female parent.

Problems and countermeasures in hybrid seed production.   A good hybrid seed production scheme has the following attributes: excellent purity of parental stocks; strong self-incompatibility of both parents; synchronized bolting and flowering of the inbreds; minimal disease and pest problems in the seed production plot; excellent isolation from possible contaminants;

and good climate for optimum expression of incompatibility, reduced pressure from diseases, and full development of seeds. Any deviation from the above scheme will almost certainly lead to a high percentage of sibs (selfs) in the hybrid seed lot, far above the commercially accepted standard of hybridity and production of poor quality seeds. For this reason, seed growers must pay close attention to a number of important factors which influence sibbing rate and seed quality.

Admixtures and off-types of parental lines: Ideally, the inbred parents should be uniform, homozygous and pure. Inbreeding and bud pollination can cause, however, certain biological disturbances such as heteroploids and other abnormalities (Iizuka 1960) which eventually impair the purity of inbred lines and contaminate the hybrid seed lot. When inbreds are multiplied on a large scale for eventual use in hybrid production, inadvertent mixtures, contamination, and occasional spontaneous genetic change may occur.

In order that such aberrants do not contribute to the F_1 hybrid seed lot, seed growers must be adequately familiar with the general characteristics of inbred lines so that deviants from normal morphology can be eliminated as early as the seedling stage, or in the seed production field before they flower and start to contribute pollen grains to other flowering plants. The parental lines in the seed production plots should be inspected several times to insure that off-types with delayed character expression are rogued out long before flowering ensues, and until one is reasonably sure that all off-types have been eliminated. Some off-types can be recognized only during extended vegetative growth. To effectively rogue out such off-types, it is necessary to observe the whole heading process of the lines. Only plants that are true-to-type are then vernalized. Although an expensive procedure, this practice is recommended to replenish the foundation stocks of the parental lines. A common method to reduce the above-mentioned problems is to minimize the frequency of inbred multiplication. This can be done only if good seed storage facilities are available.

Weak incompatibility system: Ideally, both inbred stocks should have strong self-incompatibility. Nonetheless, some hybrids in which one or both parents have less than the ideal incompatibility strength may be multiplied, as long as the good combining ability of parents offsets these limitations.

In the case where only one parent is strongly self-incompatible, hybrid seeds are harvested only from that strongly self-incompatible parent. However, this leads to reduced productivity and ultimately, to a higher seed cost.

The second case, i.e. neither parents are strongly self-incompatible, is difficult to overcome and is rarely encountered since breeders often select for strong incompatibility. This type of combination is generally not pursued except in special circumstances. To minimize the sib (self) production, extra efforts and attention must be given to insure that the peak pollination period occurs at an optimum temperature and humidity so that the self-incompatibility expressions of both parents are at their highest levels.

Row ratios of parent inbreds may also be modified to conform with the most ideal arrangement (Fig. 32). If both parent lines carry strong self-incompatibility and cross-fertility, they are normally planted alternately (1:1 ratio). However, parent lines may differ in cross-fertility and pseudo-fertility (open flower selfing or sibbing). In this case the appropriate ratio will have to be carefully decided (often a responsibility of the breeder) and seed growers should follow the prescribed ratio.

As an example, a situation may be visualized wherein one line has a much stronger incompatibility than the other line. A modified ratio of two rows of the strongly incompatible line to one row of the weakly incompatible line may be possible. In such an arrangement, honeybee foraging among plants of the weakly incompatible line would be minimized while at the same time enhancing cross pollination in its direction. Bee activity among sib plants of the strongly incompatible line will, of course, increase but the strength of incompatibility should minimize the sibbing percentage in the eventual hybrid seed lot.

Asynchrony of flowering: The parent inbred lines must flower at or about the same time to maximize the pollen exchange between them. When the peak of flowering of parent lines
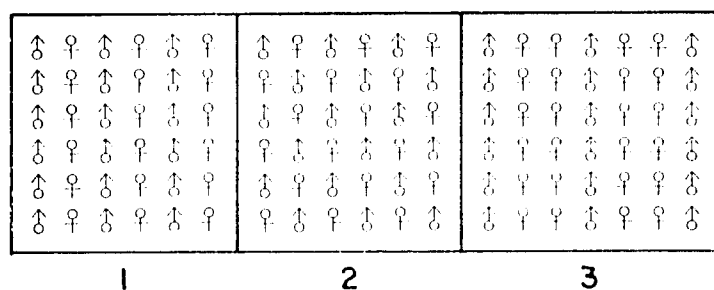
Figure 32. Planting methods of two parental lines for hybrid seed
production in Chinese cabbage: (1) alternate line method
with 1:1 ratio; (2) alternate plant method with 1:1 ratio;
and (3) alternate line method with seed plant:pollen plant
at 2:1 ratio.

occurs at different periods, the sibbing rate in the hybrid seed lot will tend to be high because
of the more frequent bee visitation between plants of the same line. This is especially true if
self-incompatibility of parent lines is not particularly strong.

The selection of inbred lines with similar bolting habits is essential in a hybrid program.
However, this is sometimes difficult to achieve since breeders are interested also in other traits.
If one line is more sensitive than the other, this line should be sown later or should be given
a shorter period of artificial vernalization. The differences in sowing date and treatment period
between the two parents should be established from repeated experiments as natural cold conditions
do not remain static; instead they fluctuate within certain limits. While adequate at times, this
synchronization method is far from perfect.

Several techniques which could aid the synchronization of flowering between lines have
been studied in the past. These treatments, which could be applied if there is any likelihood
that parent lines will flower at different times, include GA₃ spraying to hasten bolting of the
late flowering stock, spraying with growth retardant (e.g. B-995 or Alar) to delay bolting of
the fast-flowering stock, application of high N level to enhance vegetative growth and to delay
bolting in the fast-flowering stock, and pruning le   the fast-flowering stock to delay bolting
(Ali and Machado 1982, Hou 1981, Kahang   Waithaka 1981, Piluek 1985, Suge and
Takahashi 1982, Thomas 1983).

While some of the above-mentioned treatments showed definite effects in small-scale
experiments, their commercial feasibility has not been tested. Moreover, the side effects of certain
chemicals on other traits, particularly incompatibility, have not been fully explored. Ultimately,
seed producers may resort to pinching off the early-blooming flowers from the fast-flowering
line but this method is obviously labor-intensive. In radish. spraying with 150 ppm GA₃ or
continuous illumination with incandescent light at 250-500 lux is commercially applied in the
seedling nursery to promote flower stalk elongation in slow-bolting parents. A similar effect
is expected in Chinese cabbage but awaits confirmation through research.

For the same reason as above, the optimum period for planting seed production lots should
be determined for a particular locality. In some areas cold conditions may not be long enough,
while in others the optimum period for seed production may be more than sufficient. Ideally,
seed production should be done in the latter type of location. If seed production, by force of
circumstance, has to occur in critically subpar areas, seed growers must have a clear idea of
the best sowing time to minimize the problems of asynchronous flowering, breakdown of
incompatibility, etc. This information can only come from careful experimentation or, as so
often happens, through painful experience.

High incidence of softrot: Softrot is particularly singled out among the major diseases
of Chinese cabbage because it can be a severe problem for seed production in the tropical and

subtropical areas. It is reasonable to assume that any future seed production of Chinese cabbage in the tropics will have to contend with this disease.

In Taiwan the plants intended for open-field seed production are planted during part of the warm season so that by the time of the full onset of the cold period, these plants are just about at heading stage and ready to be primed for natural vernalization. If planted too early, the crop could be far advanced in its heading process when conditions for natural vernalization become suitable. When this happens, the heads could be split open and stripped of headed leaves to facilitate the emergence of the young growing point. In this situation, however, softrot attack is often debilitating. The method is also laborious. To minimize these problems, it is better to plant early in the cool season and expose the seedlings to progressively lower temperatures. Depending upon the cold conditions, these plants will generally bypass the heading stage and go directly to the flowering stage after some period of vernalization and growth. Although seed yield per plant is expectedly lower than if mature plants are induced to seed, seed yield per unit area is higher because of the low softrot incidence.

At AVRDC, where hybrid seeds are generally produced in limited quantities, germinating seeds or young seedlings of the inbred lines are vernalized artificially in the cold chamber. The softrot incidence in this seed production scheme is practically nil, except in years when the weather is unusually wet. This method is also advantageous in that cold treatment is more definitive than natural vernalization and can easily be adjusted depending upon the response of parental inbred lines. However, the amount of inbred seeds required is generally high since one needs to compensate for the resulting thrifty growth of vernalized plants (especially if they originated via seed vernalization) through high-density plantings. The application of seedling vernalization method for seed production in the tropics may be attractive, since problems of insufficient cold treatment, asynchronous flowering, softrot and other associated difficulties may be avoided. A simple household refrigerator may be modified for vernalization purposes.

In spite of the major problems associated with the sporophytic SI system (see previous sections), it can be successfully applied for commercial hybrid seed production of Chinese cabbage under tropical and subtropical conditions. The availability of uniform and high-yielding hybrid varieties of Chinese cabbage for the hot, humid tropics from AVRDC and private Taiwanese seed companies is evidence of this success.

Open-pollinated Seed Production

In the seed production of open-pollinated varieties, care should be given to maintain two essential, but somewhat contradictory, features: identity and integral heterozygosity of the variety. In order to maintain the identity of a variety, a strict selection of elite, true-to-type plants is employed in breeder seed production, while careful roguing to discard the off-types is practiced in commercial seed production. The number of selected plants used as parents, especially of breeder seeds, should be large enough to curtail any undesirable genetic drift. Fifty or more plants are generally believed to be sufficient, although there is no definite information on the critical number.

Adequate isolation from any foreign pollen sources of other cultivars and other *Brassica* species should be provided to maintain the genetic purity of the new seedlot. The destruction of any flowering *Brassicas* within 1000 m from the field is recommended for commercial seed production, whereas a more strict and reliable means, such as a screenhouse, is required for breeder seed production. Vigorous bee activity inside the screenhouse or field is essential to assure the heterozygous feature of the variety and to maximize the seed yield.

Open-pollinated varieties are usually more stringent in vernalization requirement than inbred parents of F_1 hybrid varieties. If they are not exposed to vernalization for a sufficient period, they will vary greatly in flowering time so that panmictic pollination cannot occur among the plants. Usually a prolonged vernalization at optimum temperature minimizes such a problem. In practice the specific vernalization requirements of individual varieties have to be established. It is also often necessary to discard extremely early, as well as late, bolters in the field.

Breeder seed production. The mass selection method is usually employed in the production of breeder seeds of open-pollinated varieties. Seeds are initially grown in the field as in actual market production of the variety. Elite plants selected therefrom on the basis of vegetative performance undergo the previously described head-to-seed production method. A selection rate of 10% to 20% by heading stage is usually practiced, although more selection often has to be made at the later reproductive stage. Flowering elite plants are transplanted into a screenhouse where appropriate insect pollinators are provided.

Where mass selection and head-to-seed is not practicable or is too expensive, a similar procedure can be employed in alternate generations but with more intensive roguing.

Commercial seed production. Compared to the positive and intensive selection of elite plants in breeder seed production, a passive and less intensive roguing is practiced in commercial seed production.

Breeder seeds are sown and vernalized by either natural or artificially low temperature, depending upon the situation. In the temperate zone, direct sowing or transplanting is done when the low temperature period is still long enough to induce a full vernalization of the particular variety. The subsequent warm temperatures and increasing daylength of the spring season provide ideal environments for flower stalk elongation, flowering and seed set. As an alternative for the tropics, seedlings could be vernalized at high altitudes (Fig. 25) and then brought down to the warmer conditions of the lower elevations for flowering and seed production. This would remove many problems of the seed-vernalization treatment such as low survival rate and poor plant vigor, and would ultimately increase seed yield per unit area. However, this possibility remains to be studied.

Continuous roguing has to be practiced to discard off-types for vegetative and reproductive traits. Plants with aberrant bolting or flowering dates should be discarded. It is advisable to remove extremely vigorous or weak plants. Very vigorous plants are often products of outcrossing. Whenever possible, roguing should be combined with thinning procedures.

At the bolting stage prior to flowering, a thorough inspection of the seed production site is necessary in order to remove all possible sources of contaminating pollen.

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Glossary

Abscission: The process by which plant parts, such as leaves and flowers, are shed off.

Adaptation: The process by which individuals (or parts of individuals), populations, or species change in form or function in such a way to better survive under given environmental conditions; also the result of this process.

Allele or allelomorph: One of a pair or series of forms of a gene which are alternative in inheritance because they are situated at the same locus in homologous chromosomes.

Allogamy: Cross-fertilization.

Amphidiploid. A polyploid whose chromosome complement is made up of the entire somatic complements of its two parental species.

Androecium: Male reproductive organs of a plant; stamens taken collectively.

Anther: The pollen-bearing portion of the stamen.

Anthesis: The process of dehiscence of the anthers; the period of pollen distribution; the period of flower opening.

Antigen: A foreign substance, usually protein or protein-polysaccharide complex in nature, which elicits the formation of specific antibodies within an organism.

Autotrophic: Being self-nourishing, manufacturing organic nutrients from inorganic raw materials.

Bacillus thuringiensis: A bacterium that causes disease in many insects, especially caterpillars; formulations of the bacteria are used as insecticides.

Backcross: In breeding, a cross of a hybrid to either of its parents. In genetics, a cross of a heterozygote to a homozygous recessive.

Bolt: Production and elongation of flower or seed stalks, to initiate growth of flower structure.

Breeder seed: Seed (or vegetative propagating material) increased by the originating, or sponsoring plant breeder or institution, and used as the stock seed for producing foundation seed.

Bud pollination: The artificial pollination done before the natural opening of the female flower. Usually pollens taken from naturally opened flowers are manually placed on the surface of stigmas of the forcibly opened flower buds.

Callose: An amorphous polysaccharide which gives glucose on hydrolysis, usually formed on sieve plates or pollen grain walls. Callose exhibits fluorescence after labelling with fluorescent compounds, which usually characterizes pollen tubes from neighboring tissues.

Campylotropous: (Of ovule) curved over so that funicle appears to be attached to the side, halfway between the chalaza and micropyle.

Character: The expression of a gene as revealed in the phenotype.

Codominance: Two alleles in a locus being equally dominant so that both alleles show their effect.

Combining ability: General, average performance of a strain in a series of crosses. Specific, deviation of performance of a given cross from that predicted on the basis of the general combining ability.

Composite: A mixture of genotypes from several sources, maintained by open pollination.

Conduplicate: State of two cotyledons being folded to embrace the radicle.

Cotyledons: Leaves formed within the seed and present on seedlings immediately after germination; seed leaves.

Crucifer: A plant in the family Cruciferae also called the mustard family, which includes the Chinese cabbage.

Cuticle: A layer of waxy material, cutin, on the outer wall of the epidermis of epidermal cell walls in plants, making them fairly impermeable to water.

Diallel cross, complete: The crossing in all possible combinations among a series of genotypes.

Dioecy (dioecious): Hybridity mechanism in which staminate and pistillate flowers occur exclusively on different individuals of the same species.

Diploid: Having two sets (genomes) of chromosomes; chromosome number of 2n, as in a zygote. Somatic or body tissue is normally diploid in contrast to haploid germ cells.

Dominance: Intraallelic interaction such that one allele manifests itself more or less, when heterozygous, over its alternative allele.

Dormancy: A state of inactivity or prolonged rest.

Egg: The female gamete or germ cell.

Emasculation: Physical removal of the anthers from a bud or flower before pollen is shed. It is a normal preliminary step in crossing to prevent self-pollination.

Embryo: The rudimentary plant in a seed; the developing product of fertilization of an egg (zygote).

Endosperm: Triploid tissue which arises from the triple fusion of a sperm nucleus with two polar nuclei of the embryo sac. In seeds of certain species, the endosperm persists as a storage tissue and is used in the growth of the embryo and by the seedling during germplasm.

Epiphytotics: Of, pertaining to, or characterizing a sudden or abnormally destructive outbreak of a plant disease, usually over an extended geographical area.

Family: A group of individuals directly related by descent from a common ancestor.

Fertility restoring genes: Nuclear genes that act to restore fertility in plants with male-sterile cytoplasm.

Fertilization: Union of an egg and a sperm (gametes) to form a zygote.

Field capacity: The moisture level in soil after saturation and runoff.

Foundation seed: Seed stocks increased from breeder seed, and so handled as to closely maintain the genetic identity and purity of a variety. Foundation seed is the source of certified seed, either directly or through registered seed.

Gamete: A mature reproductive cell (e.g. pollen and egg cell), capable of fusing with a cell of similar origin but of opposite sex to give a zygote.

Gametogenesis: The formation of male and female gametes, or what are called 'sex cells'.

Gametophytic self-incompatibility: Self-incompatibility system in which phenotypic expression of S-gene is determined by the allele of the very pollen or egg in question.

Gene: The unit of inheritance which is located on the chromosome; by interaction with other genes, the cytoplasm, and the environment, it affects or controls the development of a character.

Genome: A set of chromosomes corresponding to the haploid set of a species.

Genotype: The genetic makeup of an organism — the sum total of its genes, both dominant and recessive; a group of organisms with the same genetic make-up.

Gibberellins: A group of plant hormones, the most characteristic effect of which is to increase the elongation of stems in a number of kinds of higher plants.

Haploid: Having a single set (genome) of chromosomes in a cell or an individual; the reduced number (n), as in a gamete.

Heritability: Capability of being inherited; the portion of the observed variance in a progeny that is inherited.

Heritability, narrow sense: Heritability estimated from the additive portion of the genetic variance.

Heteroploid: Pertaining to chromosome numbers deviating from the normal number of the diplophase of a given species.

Heterosis (hybrid vigor): Hybrid vigor such that an F_1 hybrid falls outside the range of the parents with respect to some character or characters. Usually applied to size, rate of growth, or general thriftiness.

Heterozygous: Having unlike alleles at one or more genetic loci in homologous chromosomes (opposite of homozygous).

Heterotrophic: Of an organism or plant requiring a supply of food from its environment.

Homomorphic: The condition of having perfect flowers of only one type.

Homozygous: Having identical alleles at corresponding loci on homologous chromosomes. A plant can be homozygous at one, several, or all loci. (opposite of heterozygous).

Hybrid: The product of a cross between two parents differing in at least one Mendelian character.

Hybridization: (a) the crossing of individuals of unlike genetic constitution; (b) a method of breeding new varieties which utilizes crossing to obtain genetic recombination.

Inbred line: A pure line usually originating by self-pollination of several generations and selection; the product of inbreeding.

Inbreeding: The mating of individuals more closely related than individuals mating at random, usually by self-pollination

Incompatibility: Hybridity mechanism in which there is failure in fertilization and seed formation after self-pollination, usually due to failure of pollen tube to penetrate the stigma, or to reduced growth of the pollen tube in the stylar tissue.

Infection: The entry of a pathogen into a host and establishment of the pathogen as a parasite in the host.

Inflorescence: A flower cluster, the arrangement and mode of development of the flowers on a floral axis.

Juvenile self-compatibility: Ability of young buds in self incompatible plants to produce selfed seeds.

Larva: The immature form of an insect, such as a caterpillar, that hatches from an egg and passes through a pupal stage before becoming an adult.

Lepidoptera: The order of insects that includes butterflies and moths.

Locule: A cavity of the ovary in which ovules reside.

Male-sterility: Absence or nonfunction of pollen in flowering plants.

Male sterility, genetic: Male sterility resulting from action of specific genes.

Male sterility, cytoplasmic: Male sterility resulting from specific cytoplasmic-genic interactions.

Mass selection: A form of selection in which individual plants are selected from a source population and the next generation propagated from the aggregate of their seeds.

Monoecy (monoecious): Hybridity mechanism in which staminate and pistillate flowers are borne separately on the same plant.

Multiple alleles: A series of alleles, or alternative forms, of a gene. A normal heterozygous diploid plant would bear only two genes of an allelic series. Multiple alleles arise by repeated mutations of a gene, each mutant giving different effects.

Nectary: A nectar-secreting gland which is often located near or in flowers.

Open-pollination: Natural cross-pollination.

Ovary: The enlarged basal portion of the pistil in which the seeds are borne.

Ovule: The structure which bears the female gamete and becomes the seed after fertilization.

Panmixia: Random mating without restriction (usually extends to include random mating under the restrictions of sex or incompatibility).

Papilla: A glandular, uni- or multicellular hair on the stigma, which has relevance to the self-incompatibility reaction.

Pedicel: The stem of an individual flower or fruit.

Phenotype: Physical or external appearance of an organism as contrasted with its genetic constitution (genotype); a group of organisms with similar physical or external makeup.

Photoperiod: Length of day or period of daily illumination which is related to flowering or normal growth of a plant.

Phyllotaxis: The arrangement of leaves on an axis or stem.

Pistil: The seed-bearing organ in the flower, composed of the ovary, the style, and the stigma.

Pollen grain: The male gametophyte, originating from a microspore.

Pollen tube: A tube developing from the germinating pollen grain. The sperm cells pass through the pollen tube to reach the ovule.

Pollination: Transfer of pollen from the anther to a stigma. Self-pollination is the transfer of pollen from an anther to the stigma of the same flower or another flower on the same

plant. Cross-pollination is the transfer of pollen from an anther on one plant to a stigma in a flower on a different or genetically remote plant.

Polycross: Open pollination of a group of genotypes (generally selected) in isolation from other compatible genotypes in such a way as to promote random mating *inter se*.

Polygenes: Many genes influencing the development of a single trait; resulting in continuous variability.

Polyphyllous: State of having many leaves.

Population: A group of closely related, interbreeding plants.

Progeny test: A progeny, or groups of progenies, grown for the purpose of evaluating the genotype of the parent.

Protandry: Hybridity mechanism in which anthers mature before pistils.

Protogyny: Maturation of pistils before anthers.

Pseudo-self-compatibility: Partial seed setting following self-pollination in an otherwise self-incompatible plant.

Pure line: A strain in which all members are identical to one another and homozygous in all loci. A pure line is generally obtained by successive self fertilization.

Raceme: An inflorescence in which the main axis is elongated but the flowers are borne on pedicels that are approximately of equal length.

Recessive: The member of an allelic pair which is not expressed when the other (dominant) member occupies the homologous loci of the paired chromosome.

Recombination: Formation of new combinations of genes as a result of segregation in crosses between genetically different parents. Also the rearrangement of linked genes due to crossing-over.

Recurrent selection. A method of breeding designed to concentrate favorable genes scattered among a number of individuals by selecting in each generation among the progeny produced by matings *inter se* of the selected individuals (or their selfed progeny) of the previous generation. If two different populations under selection are mutually used as testers for the other population in the progeny test, the system is called 'reciprocal recurrent selection'.

Replum: A wall formed by ingrowths from the placenta and dividing a fruit into sections at dehiscence.

Registered seed: The progeny of breeder or foundation seed and so handled as to closely maintain the genetic identity and purity of a variety. Registered seed is the source of certified seed. Registered seed must be approved and certified by an official seed certification agency.

Rogue: A variation from the standard type of a variety or strain. Roguing, removal of undesirable individuals to purify the stock.

Rosette: A cluster of leaves radiating out from the main stem on short, overlapping stalks.

S₁, S₂, ... etc.: S_1, S_2, ... etc.: Symbols to serially designate self-incompatible alleles.

Seed: A mature fertilized ovule with its normal coverings. A seed consists of the seed coat, embryo, and, in certain plants, an endosperm.

S-gene: The gene conferring the trait of self-incompatibility.

S-proteins: Plant protein associated with self-incompatibility activation.

Selection: Any process, natural or artificial, which permits an increase in the proportion of certain genotypes or groups of genotypes in succeeding generations.

Self-incompatibility: Genetically controlled physiological hindrance to complete fertilization and seed development after self-pollination of a functional hermaphrodite.

Senile self-compatibility: Ability in aged flowers of self-incompatible plants to produce selfed seeds.

Septum: A separating partition, as in fruits.

Shattering: The fortuitous loss of seed from a plant before harvest.

Sibs, full: Progenies of the same male and female parents derived from different gametes; **half sibs**, progenies with one parent in common.

Sibbing (Sib mating): The mating between sibs.

Side-dressing: Fertilizer added to the soil around the base of a growing crop.

Silique: The fruit characteristic of Cruciferae; two-celled, the valves splitting from the bottom

and leaving the placentae with the false partition stretched between.

Sporophytic self-incompatibility: A self-incompatibility system in which the phenotypic expression of an *S*-gene is determined by the genotype of the plants from which the pollens or eggs are derived.

Stamen: The pollen-bearing organ in the flower; composed of an anther and a filament.

Stigma: The portion of the pistil which receives the pollen.

Strain: A group of individuals from a common origin. Generally a more narrowly defined group than a variety.

Style: The stalk connecting the ovary and the stigma.

Synergism: The action of two or more substances, or organisms to achieve an effect of which each is individually incapable to do or achieve.

Synthetic variety: A variety produced by crossing *inter se* a number of genotypes selected for good combining ability in all possible hybrid combinations, with subsequent maintenance of the variety by open pollination.

Tap root: The primary descending root, forming the direct continuation from the radicle.

Taxon: A general term for any taxonomic rank, from subspecific to divisional level.

Testcross: A cross of a hybrid with one of its parents, or to a genetically equivalent homozygous recessive to test for homozygosity. In breeding, testcross may also refer to crossing a selected individual with a tester stock, e.g. inbred line, variety, etc., to determine the breeding value of that individual (see also topcross).

Tetradynamous: Pertains to the male organ structure in the flower having four long anthers and two short ones.

Thorax: The second of three major divisions in the body of an insect, the one bearing the legs and wings.

Topcross: A cross between a selection, line, clone, etc., and a common pollen parent which may be a variety, inbred line, single cross, etc. The common pollen parent is called the topcross or tester parent (see also testcross).

Trait: A distinctive definable characteristic; the mark of an individual plant.

Trinucleate pollen: Pollen having two reproductive and one vegetative nucleus when released from the anther.

True leaf: Any leaf produced after the cotyledons.

Vector: Any kind of agent, usually an insect, that carries and transmits disease-causing organisms.

Vegetative growth: Growth of stems, roots, and leaves, not of flowers and fruits.

Vernalization: The treatment of seeds before sowing or plants after sowing to hasten flowering by exposure to temperatures slightly above freezing.

Zygote: A diploid cell formed by the fusion of two haploid gametes during fertilization and usually containing two complete genomes; also the individual derived from it.

Appendix

Procedures for Small-scale Artificially Vernalized Seed Production of Chinese Cabbage Hybrid No. 62

1. Sowing of the late-flowering parental line, B-18

   Germinate B-18 seeds on moistened filter paper in 9-cm ID petri dish at 50 seeds/dish. Keep in room temperature for about 24-36 hours. It is recommended to place petri dishes upper cover down in a slanted position with a slope of about 15°. This reduces the frequency of watering and helps the plants develop a neat arrangement of growing roots.

2. Vernalization of B-18

   Two days after sowing of B-18, transfer the petri dishes to 5° to 10°C cold room with continuous artificial dim light. Water regularly with distilled water.

3. Sowing of the early-flowering parental line, E-7

   Seven days after sowing of B-18, sow seeds of E-7 following the same manner as for B-18. Keep in room temperature for about 24-36 hours.

4. Vernalization of E-7

   Eight days after sowing of B-18, transfer petri dishes with E-7 to 5°-10°C cold room with continuous artificial dim light.

5. Pricking

   Four weeks after sowing of B-18, take petri dishes (B-18 and E-7) out of the cold room and keep them in a lighted room at room temperature for 5-6 hours. Before pricking water until seedlings and filter paper are thoroughly moistened in order to avoid root damage when detaching the seedlings from the filter paper. Prick in flats (with spacing of about 7 cm between rows and between plants) or small pots and keep under partially shaded area until seedlings are fully recovered. Cover the flats or pots with fine mesh screen (nylon net) to decrease insect damage and intense sunlight during the early stage of growth. Water periodically (1-3 times a day) to maintain optimum moisture.

6. Transplanting

   Three to four weeks after pricking, transplant seedlings to an isolated field, inside nethouse or in medium-sized clay pots containing soil:sand:compost and rice hull at a ratio of 5:1:3:1. Transplanting date depends on the vigor of seedlings. A spacing of 50-60 cm between rows and 20-30 cm between hills is recommended. The application of compost, lime and borax in addition to NPK is recommended as basal-applied fertilizers under most soil conditions. The suggested rate of NPK application is about one-third to one-half of that needed for head production. Sidedressings, with small amounts of NPK at bolting and flowering stage, are

desirable. At AVRDC we split the application several times (mostly once every two weeks after transplanting through the time of bee release). Field arrangement of two parents in a 1:1 ratio is recommended.

7. Pinching and enclosing

Three weeks after transplanting, pinch the terminal buds of the main flower stalks to encourage more and vigorous lateral flower stalk formation. Enclose plots with insect cages if plants have been growing in a nonisolated, open field.

8. Bee pollination

About ten days after pinching and enclosing, if both parental lines flower synchronously and profusely for pollination, honey bees can be released in the cage. In caged pollination the asynchronous flowering problem may be controlled by delaying the introduction of honey bees and/or by eventual removal of old flowers/setting pods from the early flowering parent. Once flowering time of both parental lines is properly synchronized, the normal procedures listed above may be followed. Asynchronous flowering under open-field conditions is much more difficult to handle and requires great labor expense. Thus, it is essential that flowering of the two parents in the field is properly matched. Close the bee box for one day and two nights before finally releasing the bees to avoid any possible pollen contamination. Pinch off all old flowers and setting pods before bee introduction. Ensure that flowering branches do not rest against the wall of the cages because honey bees outside sometimes forage such flowers leading to contamination. At this stage careful attention should be paid to minimize contamination. The cleaning of hands and clothes of any person entering the cage is required. Under open-field conditions, removal of flowering crucifers nearby is mandatory. Any flowering Chinese cabbage plant within 1,000 m radius from the seed-production field must be removed.

9. Bee removal

Three to four weeks after bee pollination, when flowering is almost over in one of two parental lines, remove the bees from the cage. Five days after the removal of bees, remove all late-blooming flowers of both parental lines

10. Harvesting

All flowers which had bloomed and had been pollinated before bee removal will develop small siliques within five days. When about 80% of the siliques have turned brown yellow, reap two parental lines separately at the bottom. Dry the harvested plants about five days under the sun, then thresh and clean the seeds. If data collection is needed on seed production, take 20 random plants of each parental line. Count the number of siliques/plant, the number of seeds/silique, the seed weight/plant, etc.

11. Processing

Threshed seeds must be dried again until the moisture content is reduced to about 7%. Sun-drying is better than forced-air drying. If the latter is used, air temperature should not be higher than 40°C. For long-term storage, it is recommended that seeds contain 6% or less moisture.

12. Sibbing rate test

Take a random sample of 500 seeds harvested from each parental line and sow in the flats, at 2 seeds per hill, with a spacing of 5 cm between rows and hills. Count selfs or sibs based on the ger   morphology of seedlings (or plants) arising from seeds harvested from E-7. This method requires a great deal of experience. In order to facilitate this test, it is

recommended that a few E-7 plants be available for comparison, preferably at a similar growthstage as seedlings or plants from the 'hybrid' seed lot. With seeds harvested from B-18, any hairless plant can be counted as sib- or selfed-plants. Hairiness is a single dominant gene. E-7 is a homozygous, hairy plant, whereas, B-18 is hairless. Sowing parental lines along with the hybrid seed lot is recommended for the comparison of general morphology.

If seeds from both parental lines prove to have high purity (99% to 100% hybrid), blending of the two seedlots is possible since, based on previous studies at AVRDC, they are not very different from each other in most horticultural traits.

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